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Jianbo Xiao
Satyajit D. Sarker
Yoshinori Asakawa
Editors

Handbook of Dietary Phytochemicals

Springer

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Jianbo Xiao • Satyajit D. Sarker •
Yoshinori Asakawa
Editors

Handbook of Dietary Phytochemicals

With 343 Figures and 139 Tables

Springer

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Preface

Phytochemicals are non-nutritive compounds that have properties to protect health, preventing diseases. They are not essential nutrients, which means that the body does not need them to sustain life. It's well known that plants produce these chemicals to protect themselves, but recent studies have shown that they can protect people from disease too. There are many thousands of known phytochemicals. Some known phytochemicals are lycopene in tomatoes, isoflavones in soybeans, and flavonoids in fruits.

Phytochemicals have existed as long as plants have, but we only know of their existence for about a hundred years. Medicinal plants are used traditionally all over the world. Knowledge of traditional medicine may have developed over the centuries by trial and error. The Chinese have the oldest medical system. Over 5,000 years ago, Chinese medicine was based on the influence of yin and yang and the five ingredients. The first mentions of herbal medicine come from 2800 BC when the Chinese Emperor Shennong wrote the words "The Great Native Herbal." Hippocrates (460–377 B.C.E.) and Aristotle (384–322 B.C.E.) brought herbs from India and Egypt to Europe. The Greek physician Dioscorides wrote "De Materia Medica" in the first century C.E.

In the nineteenth and twentieth centuries, the main goal of scientists was to discover active ingredients with medicinal or insecticidal properties. Results of such efforts were the discovery of salicylic acid, morphine, and pyrethroids. In the 1980s, many laboratories began identifying phytochemicals in plants that could be used as drugs. Many of the phytochemicals discovered appear to prevent or help against cancer, heart disease, and stroke. At the same time, other scientists were conducting epidemiological studies to determine the relationship between the consumption of certain phytochemicals and human health. Most studies show that a diet rich in plants reduces the incidence of cancer and heart disease.

Currently, most new drugs are not found in plants, but they are newly synthesized products. Recently, we have taken a new interest in the discovery of phytochemicals. What regains attention is the fact that many chemicals have not yet been discovered. New, state-of-the-art laboratory technology facilitates the discovery and identification of new phytochemicals.

The *Handbook of Dietary Phytochemicals* summarizes the latest advances in the chemistry, biological activity, nutrition, and function of dietary phytochemistry, as

well as the latest advances in the health and function of phytochemical-rich foods. It consists of 44 chapters that discuss the different chemical types of phytochemicals in our diet and food as well as present data collected from animal or human experiments that are directly related to human health. Each chapter covers chemistry, epidemiological studies, bioavailability, function, and safety of biological activity (animal experimentation) in humans and products on the market. In addition, over 200 figures make it easier to understand the main findings in each area. This book will become a valuable tool for researchers in food science and nutrition.

Full Professor of Nutrition and Food Science Dr. Jesus Simal-Gandara
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Prof. Jianbo Xiao is at present a professor in the Faculty of Food Science and Technology, University of Vigo, Spain. He obtained his Ph.D. in nutritional science from Okayama Prefectural University, Japan (2009). Prof. Xiao worked as a postdoc supported by the AvH Foundation at the University of Würzbug, Germany (2013–2015). Then, he worked as an assistant professor at the University of Macau from 2015 to 2020. His research focuses on dietary polyphenols. He has accepted and published more than 300 peer-reviewed papers in journals such as *Nature Reviews Drug Discovery*, *Biotechnology Advances*, *Medicinal Research Reviews*, *Trends in Food Science & Technology*, *Critical Reviews in Food Science and Nutrition*, *Food Chemistry*, *Molecular Nutrition and Food Research*, and so on (Google scholar citation:11000, H-index=56). Prof. Xiao was selected as 2016, 2017, 2019, and 2020 Clarivate Analytics Highly Cited Researcher (HCR) in agricultural science, and he is currently "the editor in chief of *Food Frontiers* (Wiley), special content editor of *Food Chemistry* and associate editor of *Journal of Berry Research, Frontiers in Pharmacology* and *Phytomedicine Plus*, and the editorial board member of *Trends in Food Science & Technology , Critical Reviews in Food Science and Nutrition, Journal of Nutritional Biochemistry, Food and Chemical Toxicology, Phytomedicine, Industrial Crops and Products, Environmental Toxicology and Pharmacology, Current*

Drug Metabolism, Food Science and Human Wellness, and so on." He was chairman of the 2015 International Symposium on Phytochemicals in Medicine and Food (ISPMF2015, Shanghai) organized by the Phytochemical Society of Europe, and its second edition (2-ISPMF, Fuzhou, 2017), third edition (3-ISPMF, Kunming, 2018), and fourth edition (4-ISPMF, Xi'an, 2020).

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Prof. Satyajit D. Sarker is Professor of Pharmacy and the Director of School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University (LJMU), Liverpool, UK. He is the Founding Head of the Centre for Natural Products Discovery at LJMU. He obtained his B.Pharm. (Hons.) and M.Pharm. (postgraduate) degrees from the University of Dhaka and his Ph.D. in Phytochemistry from the University of Strathclyde, Glasgow, UK. Prof. Sarker is a world-renowned natural products researcher, whose research falls in the area of pharmaceutical and natural products chemistry and rational drug design (medicinal chemistry), and focuses on anticancer, analgesic, anti-inflammatory, antioxidant, antimalarial, and antimicrobial properties, and cancer chemopreventive and wound-healing potential of purified compounds from higher plants as well as novel synthetic organic molecules.

Prof. Sarker is one of the most cited phytochemists and has over 630 publications with more than 18,800 citations, an h-index of 61, and an i10 index of 401 (Google Scholar). He has been the Editor-in-Chief of *Phytochemical Analysis* since 2010, has been part of the Editorial Advisory Board of more than 40 journals, and regularly reviews articles for more than 80 international journals. He coauthored the popular textbook, *Chemistry for Pharmacy Students*, published by Wiley & Sons in 2007, and subsequently, this book was translated in Indonesian, Japanese, Greek, and Portuguese languages; the second edition of this book was published in 2019. He is also the coauthor of the only book on steroid dimers, published by the same publisher in 2012. He coedited both the second and third editions of the book, *Natural Products Isolation*, published by Humana Press

Springer-Verlag, respectively, in 2005 and 2012. His novel contribution in the field of phytochemistry is the book *Computational Phytochemistry*, published by Elsevier in 2018, and his latest book is *Medicinal Natural Products: A Disease-Focused Approach*, published by Elsevier in 2020.

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Prof. Dr. D.H.C. Yoshinori Asakawa first studied biology at the Tokushima University, then went to graduate school at Hiroshima University in 1964 and studied organic chemistry, and obtained his PhD degree there and was appointed as a research assistant. Then, Prof. Asakawa went to the Université Louis Pasteur, France, as a postdoctoral fellow, where he worked for 2 years (1972–1974) with Prof. Guy Ourisson, one of the most original natural product chemists worldwide. In 1976 he moved to Tokushima Bunri University as an associate professor, and was promoted to full professor in 1981. Prof. Asakawa served as dean twice, and since 1986, he is director of the Institute of Pharmacology.

Prof. Asakawa is the former editor of *Phytomedicine and Spectroscopy,* and serves on the editorial boards of the *Journal of Natural Products, Phytochemistry*, *Phytochemistry Letters*, *Planta Medica*, Current Chemistry and Biology, *Fitoterapia*, *Flavour and Fragrance Journal*, *Natural Product Research*, *Natural Product Communications, Arkivoc and Malaysian Journal of Sciences*, among others. He is the president of the Phytochemical Society of Asia (PSA) and of Chemistry of Terpenes, Essential Oils, and Aromatics (TEAC) and permanent committee member of ISEO.

His research interests are the isolation and structure elucidation of bioactive secondary metabolites of bryophytes, pteridophytes, and inedible mushrooms; medicinal and aromatic plants and insects and their bioassay; biotransformation of secondary metabolites by fungi and mammals; total synthesis of natural products; chemical reaction of organic per-acids; and chemical phylogeny of spore-forming plants. Prof. Asakawa has published, up to now, 700 original papers, 14 reviews,

and 40 books. His three books on the "chemical constituents in bryophytes," appeared in "Progress in the Chemistry of Organic Natural Products Vol. 42 (1982), 65 (1995) and 95 (2013)" (Springer Verlag), are the "bibles" for bryophyte researchers, biochemists, phytochemists, and others.

For his outstanding research, he was awarded the Hedwig Medal by the International Association of Bryologists; the International Prize of Phytochemistry; Jack Cannon Gold Medal; International Symposium of Essential Oils Award; Gusi International Peace Prize; Gerald Blunden Award; Japanese Society of Pharmacognosy Prize; Gold Medal and Doctor Honoris Causa from Lublin Medical University, Poland; Polish Ambassador of Pharmacy and Fellow of The National Society of Ethnopharmacology, India; and the honorary membership of Turkish Academy of Science.

Since 1978, he was invited as the opening, plenary and invited lecturers from 52 countries and he invited 57 post docs and 30 students from various countries into his laboratory and organized International symposia for 8 times in his University as the chairperson.

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Introduction of Phytonutrients

1

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Abstract

Phytonutrients can be defined as phytochemicals that are present in phyto-foods and have some nutritional value, especially in relation to maintenance of human health and prevention of diseases. Their regular and adequate intake may offer protection from major chronic diseases, including cardiovascular and neurodegenerative diseases, and cancer. Phyto-foods like apples, blueberries, broccoli, cabbage, carrots, cherries, soybeans, tomatoes, and walnuts contain phytonutrients of various chemical classes. There are over 25,000 phytonutrients from different phytochemical classes, e.g., anthocyanins, carotenoids, coumarins, flavonoids, diarylalkanoids, lignans, phenolic acids, polyphenols, sterols, and terpenes, which have been found in plant foods. This chapter introduces various types of phytonutrients and their health benefits and leads to various relevant chapters incorporated in this book.

Keywords

Phytonutrients · Phytochemicals · Secondary metabolites · Phyto-food · bioactivity

1.1 Introduction

Simply, phytonutrients are phytochemicals that are present in plant-based food (also known as phyto-food) and have some nutritional value, especially in relation to maintenance of human health and prevention of human ailments. They are not necessarily essential compounds like vitamins and minerals that are found in plants, but they are usually plant secondary metabolites that help proper functioning of human body. There are over 25,000 phytonutrients discovered from plant foods, and they belong to several classes of phytochemicals, e.g., anthocyanins, carotenoids, coumarins, flavonoids, diarylalkanoids, lignans, phenolic acids, polyphenols, sterols, and terpenes. In addition to various fruits and vegetables consumed by humans, other plant-based food items, e.g., whole grains, nuts, beans and tea, also possess significant amounts of phytonutrients. The beneficial effects of these phytonutrients most often stem from their inherent antioxidative properties and may offer cancer chemoprevention, enhance cardiovascular health, and contribute to management of diabetes (Krzyzanowska et al. 2010; Probst et al. 2017; Bansal et al. 2018). Although most phytonutrients do not work like drugs to treat diseases, epidemiological evidence suggests that phytonutrients intake can be linked to a number of positive health outcomes including reduced risk for chronic diseases (Probst et al. 2017). While the book, *Handbook of Dietary Phytochemicals*, comprises more than 40 chapters covering various classes of dietary phytochemicals mainly in the context of their beneficial role to human health, this chapter is aimed to briefly introduce various aspects of phytonutrients or dietary phytochemicals, highlighting their major sources, chemical classes, and major health benefits.

1.2 Major Dietary Sources of Phytonutrients

The major sources of dietary phytonutrients are mainly fruits and vegetables, but whole grains, nuts, beans, and tea, which are present in our regular diets, can be good sources of phytonutrients. Plants producing edible fruits, vegetables, and spices that contain significant amounts of phytonutrients belong to a number of families. The families, Apiaceae (*alt.* Umbelliferae), Cruciferae, Cucurbitaceae, Fabaceae (*alt.* Leguminosae), Rosaceae, and Rutaceae, are just six examples of such plant families. Some examples of common sources of phytonutrients are presented in Table 1.

Table 1 Examples of some phytonutrients and their main sources

Phytonutrients	Sources		
	Common name	Botanical name	Family
Anthocyanins	Aubergine	*Solanum melongena* L.	Solanaceae
	Blackcurrant	*Ribes nigrum* L.	Grossulariaceae
	Blackberries	*Vaccinium* Sect. *cyanococcus* Rydb.	Ericaceae
	Blueberries	*Vaccinium* Sect. *cyanococcus* Rydb.	
	Cranberries	*Vaccinium* Subg. *oxycoccus* L.	
	Red cabbage	A cultivar of *Brassica oleraceae* L.	Brassicaceae
Carotenoids	Carrots	*Daucus carota* L.	Apiaceae
	Pumpkin	*Cucurbita pepo* L.	Cucurbitaceae
	Tomatoes	*Solanum lycopersicum* L.	Solanaceae
	Watermelons	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	*Vaccinium* Sect. *cyanococcus* Rydb.
Cinnamic acid derivatives	Aubergine	*Solanum melongena* L.	Solanaceae
	Kiwi fruits	*Actinidia deliciosa* L.	Actinidiaceae
	Plums	*Prunus* Subs. *Prunus* L.	Rosaceae
Coumarins	Anise	*Pimpinella anisum* L.	Apiaceae
	Caraway	*Carum carvi* L.	
	Carrots	*Daucus carota* L.	
	Lemon	*Citrus limon* (L.) Burn. f.	Rutaceae
	Orange	*Citrus sinensis* (L.) Osbeck	
Ellagic acid	Pomegranates	*Punica granatum* L.	Lythraceae
	Raspberries	*Rubus idaeus* L.	Rosaceae
	Strawberries	*Fragaria* x *ananassa* Duchesne	

(continued)

Table 1 (continued)

Phytonutrients	Sources		
	Common name	Botanical name	Family
Flavonoids	Apples	*Malus pumila* Miller	Rosaceae
	Citrus fruits (e.g., lime)	*Citrus aurantifolia* (Christm.) Swingle	Rutaceae
	Green tea	*Camellia sinensis* (L.) Kuntze	Theaceae
	Kale	*Brassica oleraceae* L.	Brassicaceae
	Onions	*Allium cepa* L.	Amaryllidaceae
Glucosinolates	Brussels sprouts	Cultivars of *Brassica oleraceae* L.	Brassicaceae
	Cabbage		
	Kale		
Resveratrol	Blueberries	*Vaccinium* Sect. *cyanococcus* Rydb.	Ericaceae
	Cranberries	*Vaccinium* Subg. *oxycoccus* L.	
	Grapes	*Vitis vinifera* L.	Vitaceae
Phenolic acids (benzoic acid derivatives)	Blackcurrant	*Ribes nigrum* L.	Grossulariaceae
	Black radish	*Raphanus sativus* L.	Brassicaceae
	Blueberries	*Vaccinium* Sect. *cyanococcus* Rydb.	Ericaceae
	Cherries	*Prunus avium* L.	Rosaceae
	Green tea	*Camellia sinensis* (L.) Kuntze	Theaceae
	Onions	*Allium cepa* L.	Amaryllidaceae
	Plums	*Prunus* Subs. *Prunus* L.	Rosaceae
Phytoestrogens (isoflavones and lignans)	Flaxseeds	*Linum usitatissimum* L.	Linaceae
	Sesame seeds	*Sesamum indicum* L.	Pedaliaceae
	Soybeans	*Glycine max* (L.) Merr	Fabaceae
Sterols	Almonds	*Prunus amygdalus* (Mill.) D. A. Webb	Rosaceae
	Brussels sprouts	A cultivars of *Brassica oleraceae* L.	Brassicaceae
	Corn or maize	*Zea mays* L.	Poaceae
	Flaxseeds	*Linum usitatissimum* L.	Linaceae
	Pistachios	*Pistacia vera* L.	Anacardiaceae
	Strawberries	*Fragaria* x *ananassa* Duchesne	Rosaceae

Dietary sources of phytonutrients are described in more details in all subsequent *chapters* dealing with various chemical classes of phytonutrients, e.g., anthocyanins, coumarins, diarylalkanoids, flavonoids, phenylpropane derivatives, and so on.

1.3 Major Classes of Dietary Phytonutrients and Their Health Benefits

Phytonutrients found in human diets are essential plant secondary metabolites, and they belong to various phytochemical classes. This section will present an overview only on the major phytochemical classes, e.g., anthocyanins, carotenoids, coumarins, diarylalkanoids, flavonoids and isoflavonoids, lignans and neolignans, phenolic acids, polyphenols (tannins), sterols and terpenoids, and their health benefits.

1.3.1 Anthocyanins

Anthocyanins are naturally occurring water-soluble pigments, which are present in many edible fruits and vegetables, and they constitute a major group of phytonutrients (Khoo et al. 2017). Well over 600 natural anthocyanins have been reported to date, all coming from six anthocyanidin aglycones derived from the flavylium backbone (**1**) (Fig. 1) with different glycosylations and acylations. Sometimes anthocyanins are considered as flavonoids, but unlike other flavonoids, anthocyanins have a positive charge on the oxygen atom of the C ring of a basic flavonoid structure.

Depending on their pH, the anthocyanin pigments can be red, purple, blue, or black. These compounds are fairly unstable, and the integrity of their structure depends on pH, light, and temperature. The *Vaccinium* species like blueberry (*Vaccinium* Sect. *cyanococcus*), cranberry (*Vaccinium* Subg. *oxycoccus*), bilberry (*V. myrtillus*), and other berries like black and red raspberry (*Rubus occidentalis*) and blackberry (*Rubus ursinus*); other fruits, e.g., blackcurrant (*Ribes nigrum*) and cherry (*Prunus avium*); and vegetables like aubergine peel (*Solanum melongena*), red cabbage (*Brassica oleraceae*), radish (*Raphanus raphanistrum* subsp. *sativus*), beetroots (*Beta vulgaris*), and colored sweet potato (*Ipomoea batatas*) are the major phyto-foods that contain high levels of anthocyanins. One of the most common anthocyanins is cyanidin (**2**) (Fig. 1). Most of the health benefits associated with anthocyanins are because of their strong antioxidant property and their effects against oxidative stress (Khoo et al. 2017; Putta et al. 2017). These phytonutrients are good for maintaining cardiovascular health, lowering cholesterol level, enhancing glucose metabolism, controlling obesity, and preventing cancers, especially breast cancer (Yousuf et al. 2016; Li et al. 2017). Anthocyanins have also been shown to possess antidiabetic, anti-inflammatory, and neuroprotective properties. Further details on this group of phytonutrients have been furnished in the *chapter* dedicated to dietary anthocyanins in this book.

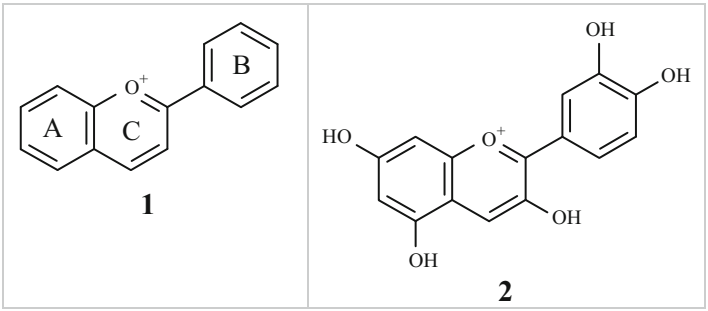


Fig. 1 The general structure of anthocyanins (**1**) and the structure of cyanidin (**2**)

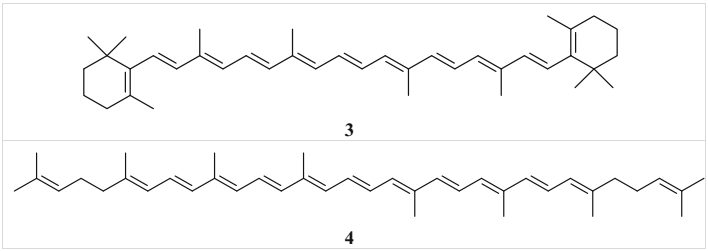


Fig. 2 Structures of β-carotene (**3**) and lycopene (**4**)

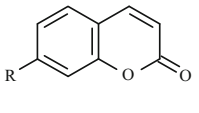
1.3.2 Carotenoids

Carotenoids are actually naturally occurring colored terpenoids, called tetraterpenoids (Fig. 2). They are yellow, orange, and red pigments produced by various plants, algae, bacteria, and fungi (Sarker and Nahar 2007a). For example, the orange color pigment found in carrots is known as β-carotene (**3**), and the red color found in tomatoes is lycopene (**4**) (Fig. 2). There are more than 700 carotenoids identified to date, about 40 of them are present in human diets. They are present extensively in various edible plants, e.g., carrots (*Daucus carota*), pumpkins (*Cucurbita pepo*), corn (*Zea mays*), and tomatoes (*Solanum lycopersicum*), and are another important group of phytonutrients with significant health benefits.

These phytonutrients possess high levels of antioxidant property, and thus the dietary carotenoids provide health benefits in decreasing the risk of various diseases, particularly cancers and cardiovascular and eye diseases (Johnson 2002; Krinsky and Johnson 2005; Saini et al. 2015; Eggersdorfer and Wyss 2018).

1.3.3 Coumarins

Coumarins are the largest class of 1-benzopyran derivatives ubiquitously distributed in the plant kingdom (Sarker and Nahar 2017), and there are about



Coumarins	R
Coumarin (**5**)	H
Umbelliferone (**6**)	OH

Fig. 3 Structures of coumarin (**5**) and 7-hydroxycoumarin (umbelliferone, **6**)

4000 naturally occurring coumarins identified to date. They also constitute one of the major groups of phytonutrients found in plant-based food items. Coumarin (**5**) (2*H*-1-benzopyran-2-one) (Fig. 3), the first member of the coumarin class, is a fragrant colorless compound, which was isolated from the tonka bean (*Dipteryx odorata*, family: Fabaceae) in 1820. The simplest member of natural coumarins is 7-hydroxycoumarin or umbelliferone (**6**), which is also the starting material for the biosynthesis of other more complex coumarins (Fig. 3). Plants from the families Apiaceae, Asteraceae, and Rutaceae are major sources of naturally occurring coumarins (Sarker and Nahar 2007a). Some commonly consumed coumarin-containing phyto-foods include apricots (*Prunus armeniaca*), carrots (*Daucus carota*), celery (*Apium graveolens*), cherries (*Prunus avium*), citrus fruits (*Citrus spp.*), parsnip (*Pastinaca sativa*), and strawberries (*Fragaria* × *ananassa*), as well as spices like aniseed (*Pimpinella anisum*), caraway (*Carum carvi*), cinnamon (*Cassia cinnamon*), coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), and fennel (*Foeniculum vulgare*). Natural coumarins can be classified into simple, simple prenylated, simple geranylated, furano, pyrano, sesquiterpenyl, and oligomeric coumarins (Sarker and Nahar 2007a, 2017).

Dietary coumarins offer various medicinal values, which are based predominantly on their antimicrobial, antiviral, anti-inflammatory, antidiabetic, antioxidant, and various enzyme-inhibitory activities. In addition to their therapeutic properties, the aromatic properties of coumarins, especially in spices, are exploited extensively in culinary, cosmetic, and tobacco industries. In this book, a whole chapter has been dedicated on coumarins as dietary phytochemicals covering various aspects of this important group of phytonutrients.

1.3.4 Diarylalkanoids

Diarylalkanoids are compounds, where there are two aryl groups (benzene rings) connected by an alkyl chain (Atta-ur-Rahman 2002). The best-known example of this group of compounds occurring in phyto-foods is curcumin (**7**) (Fig. 4), which is a diarylheptanoid that occurs in the spice/food color called turmeric obtained from the rhizomes of *Curcuma longa* (Sarker and Nahar 2007b; Hewlings and Kalman 2017). Another popular example is resveratrol (**8**) (Fig. 4), which is

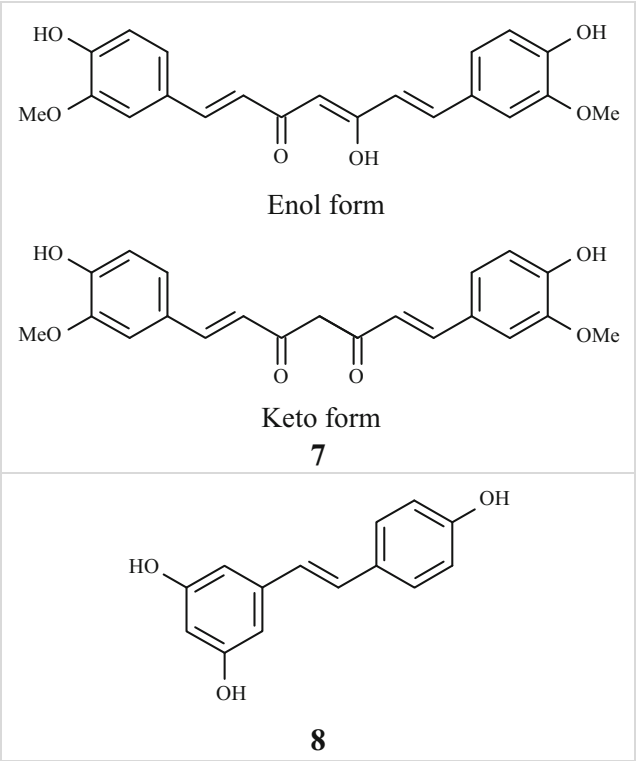


Fig. 4 The structures of curcumin (**7**) and resveratrol (**8**)

actually a diarylethanoid, and found in high quantities in red grapes (*Vitis vinifera*) (Salehi et al. 2018).

Both curcumin (**7**) and resveratrol (**8**) are well-known natural antioxidants and have been linked to a plethora of health benefits, including management of arthritis, inflammation, protection against cancer, heart diseases, and diabetes (Hewlings and Kalman 2017; Salehi et al. 2018).

1.3.5 Flavonoids and Isoflavonoids

Flavonoids are the derivatives of 1,3-diphenylpropane, and they constitute a large group of phytonutrients, which are widespread in various fruits, vegetables, and edible flowers (Sarker and Nahar 2007a). Most flavonoids are yellow pigments and contribute to the color of the flowers and fruits where they usually occur as *O*- or *C*-glycosides. While quercetin (**9**) is the most common flavonoid found in phyto-foods, rutin (**10**) and hesperidin (**11**) are the most prevalent of all flavonoid glycosides found in fruits and vegetables (Fig. 5). There are well over 2,000 glycosides of the flavones and flavonols classes reported in the literature

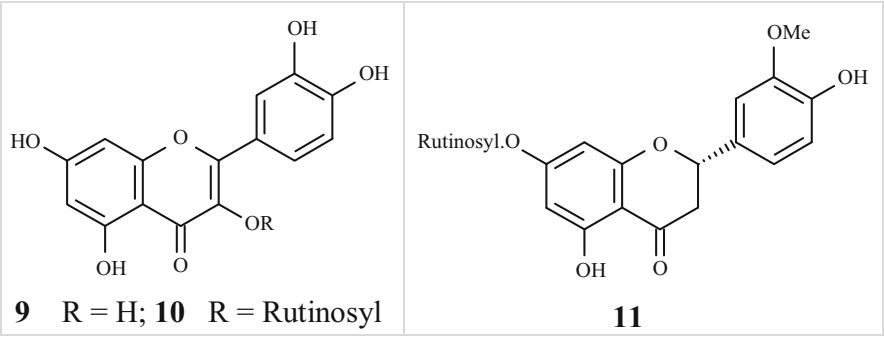
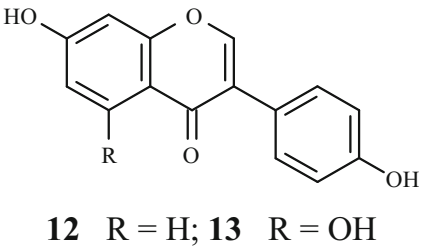


Fig. 5 The structures of quercetin (**9**), rutin (**10**), and hesperidin (**11**)

Fig. 6 The structures of daidzein (**12**) and genistein (**13**)



(Sarker and Nahar 2007a). The major dietary sources of flavonoids include tea (*Camellia sinensis*), citrus fruits (*Citrus* species, e.g., *Citrus sinensis*), apples (*Malus pumila*), and legumes (from the Fabaceae family) (Yao et al. 2004).

Most flavonoids are potent antioxidants, because of their high propensity to electron transfer, ferrous ions chelating activity, and direct scavenging of reactive oxygen species. Several flavonoids possess anticancer, antiaging, anti-inflammatory, antihepatotoxic (hepatoprotective), antitumor, antimicrobial, antithrombotic, and antiviral properties (Yao et al. 2004; Sarker and Nahar 2007a). The antioxidant properties of flavonoids present in fresh fruits and vegetables usually contribute to their preventative effect against cancer and heart diseases.

When the 2-phenyl side chain of flavonoid isomerises to the 3-position, it gives rise of a new class of bioactive compounds called isoflavones, e.g., daidzein (**12**) and genistein (**13**) (Fig. 6) (Sarker and Nahar 2007a), which are part of the human diets world over, particularly in Far East Asian countries, where the traditional cuisine is usually rich in soy products that contain high amounts of isoflavonoids.

Dietary legumes (species from the family Fabaceae *alt*. Leguminosae), including soybeans (*Glycine max*), chickpeas (*Cicer arietinum*), fava beans (*Vicia fava*), pistachios (*Pistacia vera*), peanuts (*Arachis hypogaea*), and other fruits and nuts, are rich sources of isoflavonoids (Bastamante-Rangel et al. 2018). Soybeans are the richest source of isoflavones and contain large amounts of daidzein (**12**) and genistein (**13**). Soy foods and ingredients contain varying concentrations of isoflavones. Isoflavones offer several human health benefits, which are predominantly

because of their high antioxidant property. Dietary isoflavones prevents the development of specific menopause symptoms and offer protection against several chronic diseases, including cardiovascular diseases, osteoporosis, neurological impairments, and hormone-dependent cancers. There are *chapters* in this book, dedicated to different types of dietary flavonoids and isoflavonoids, which present details on outcomes from various clinical trials, and in vivo animal studies.

1.3.6 Lignans and Neolignans

Lignans, e.g., matairesinol (**14**), are another important group of phytonutrients present in our diets. They are a group of phenolic compounds, biosynthesized from the conjugation of two phenylpropane units, e.g., coniferyl alcohol (Sarker and Nahar 2007a), linking two units in different positions except direct linking of two phenyl moieties (Fig. 7). On the other hand, neolignans, e.g., magnolol (**15**), are formed from two units of phenylpropenes linking directly through two phenyl groups (Fig. 7).

Lignans and neolignans are widely distributed in the plant kingdom and particularly found in high concentrations in the plants from the families, e.g., Asteraceae, Berberidaceae, Magnoliaceae, Pinaceae, Piperacae, Phytolaccacae, and Rutaceae (Sarker and Nahar 2007a). Among the phyto-foods, flax seeds (*Linum usitatissimum*) and sesame seeds (*Sesamum indicum*) contain the highest amounts of lignans, e.g., hydroxyresinol, lariciresinol, matairesinol (**14**), pinoresinol, secolariciresinol, sesamin, and syringaresinol (Teponno et al. 2016; Rodriguez-Garcia et al. 2019). Other dietary sources of lignans include cereals like barley (*Hordeum vulgare*), oat (*Avena sativa*), and wheat (*Triticum aestivum*), soybeans, cruciferous vegetables, and some fruits, particularly apricots (*Prunus armeniaca*) and strawberries.

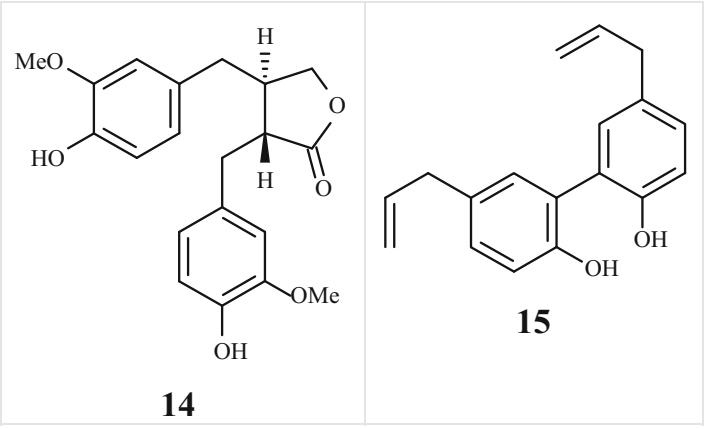


Fig. 7 The structures of the lignan, matairesinol (**14**), and the neolignan, magnolol (**15**)

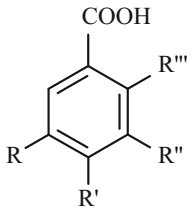
Lignans and neolignans have anticancer, antiasthma, antihypertensive, anti--inflammation, antimicrobial, antioxidant, cardioprotective, hepatoprotective, hypocholesterolemic, insecticidal, and pro-estrogenic properties (Peterson et al. 2010; Rodriguez-Garcia et al. 2019).

1.3.7 Phenolic Acids

Organic acids with phenol functionalities are usually termed as phenolic acids, and several of these compounds occur abundantly and widely in the plant kingdom. Many phyto-foods, such as fruits, vegetables, spices, nuts, and grains, possess high amounts of these compounds (Robbins 2003; Sarker and Nahar 2007a). Phenolic acids can be subdivided into two subgroups: simple benzoic acid derivatives, e.g., benzoic acid (**16**), gallic acid (**17**), salicylic acid (**18**), and vanillic acid (**19**) (Fig. 8), and cinnamic acid derivatives, which are in fact phenylpropene derivatives, e.g., caffeic acid (**20**), cinnamic acid (**21**), coumaric acid (**22**), and ferulic acid (**23**) (Fig. 9) (Natella et al. 1999; Del Olmo et al. 2017).

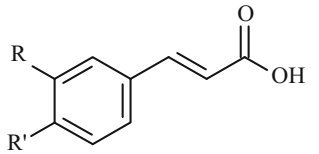
Phenolic acids constitute a large class of phytonutrients and offer significant beneficial effects on human health and well-being, particularly offering protective effects against various chronic diseases, e.g., coronary heart disease, stroke, and cancers (Del Olmo et al. 2017). Many dietary benzoic and cinnamic acid derivatives, particularly those with phenolic hydroxyl group(s), are well-known antioxidants and have several health benefits due to their strong free radical-scavenging properties (Sova 2012).

Dietary phenolic acids possess antidiabetic, anticancer, anti-inflammatory, antioxidant, and antitumor properties as well as various levels of antimicrobial properties (Natella et al. 1999; Sova 2012; Adisakwattana 2017). In this book, there are several *chapters* dealing with various aspects of different phenolic acids.



Compounds	R	R’	R’’	R’’’
16	H	H	H	H
17	OH	OH	OH	H
18	H	H	H	OH
19	OMe	OH	H	H

Fig. 8 The structures of simple benzoic acid derivatives: benzoic acid (**16**), gallic acid (**17**), salicylic acid (**18**), and vanillic acid (**19**)



Compounds	R	R’
20	OH	OH
21	H	H
22	H	OH
23	OMe	OH

Fig. 9 The structures of cinnamic acid derivatives: caffeic acid (**20**), cinnamic acid (**21**), coumaric acid (**22**), and ferulic acid (**23**)

1.3.8 Polyphenols (Tannins)

Polyphenols are naturally occurring compounds with multiple phenolic moieties (Sarker and Nahar 2007a) and distributed ubiquitously in the plant kingdom. Although this large group of phytonutrients tends to cover a variety of phenolic compounds, this section only refers to tannins, which are water-soluble heterogeneous group of polyphenols with high molecular weights ranging from 500 to 3000 Daltons (Chung et al. 1998; Smeriglio et al. 2017), found in phyto-foods, especially in fruits, e.g., apples, blackberries, dates (*Phoenix dactylifera*), grapes, mangoes (*Mangifera indica*), raspberries, strawberries; vegetables, e.g., rhubarbs (*Rheum* × *hybridum*) and squash (*Cucurbita* species); and tea (*Camellia sinensis*) (Smeriglio et al. 2017). Tannins are usually polymeric complex forms of phenolic acids, e.g., hydroxybenzoic, gallic acid (**17**), and ellagic acid (**24**), as well as phloroglucinol (**25**) and anthocyanins (Fig. 10).

Tannins can be classified into two major classes: hydrolyzable tannins and condensed tannins (or non-hydrolyzable tannins or proanthocyanidins). Hydrolyzable tannins could be further divided into gallotannins, which yield sugar and gallic acid on hydrolysis, and ellagitannins, which on hydrolysis do not produce only sugar and gallic acid, but also ellagic acid (**24**) ((Smeriglio et al. 2017).

Tannins are often present in unripe fruits but disappear during ripening. They provide plants with protection against microbial attacks. They offer beneficial effects on metabolic disorders and prevent the onset of several oxidative stress-related diseases (Smeriglio et al. 2017). They are useful in the treatment of diabetes, diarrhea, bleeding gum, and skin injuries. Tannins are strong antioxidants, and they possess anthelmintic, anticancer, antidiabetic, anti-*Helicobacter pylori*, anti-inflammatory, antimicrobial, anti-nutritional, antioxidant, anti-ulcer, antiviral, cardioprotective, and immune-regulating activities (Sarker and Nahar 2007a; Serrano et al. 2009; Kumari and Jain 2012; Smeriglio et al. 2017).

1.3.9 Sterols

Phytosterols are a group of naturally occurring 3-hydroxysteroids, widely distributed in the plant kingdom, and many phyto-foods are rich in phytosterols, e.g.,

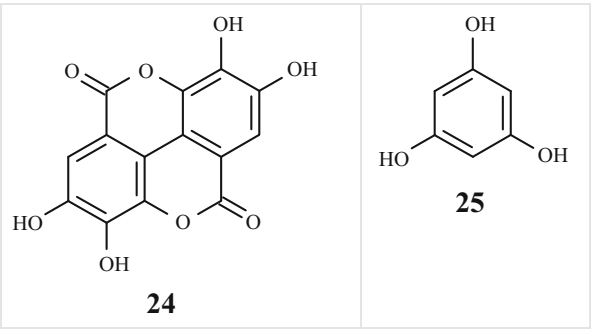


Fig. 10 The structures of ellagic acid (**24**) and phloroglucinol (**25**)

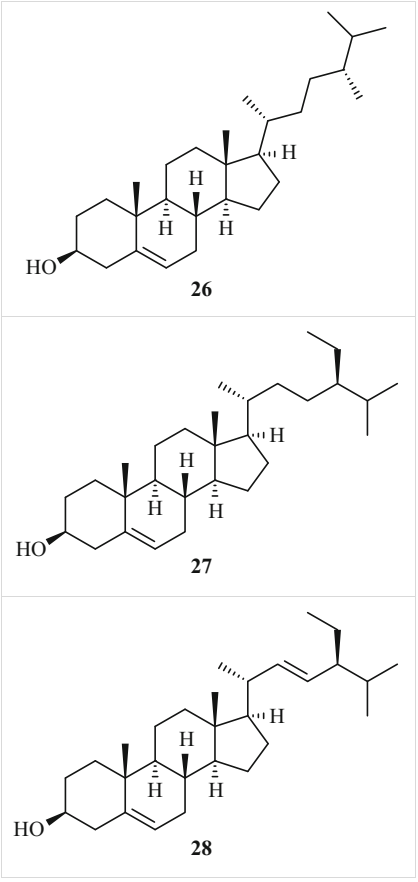


Fig. 11 The structures of major dietary phytosterols: campesterol (**26**), β-sitosterol (**27**), and stigmasterol (**28**)

campesterol (**26**) (sources: banana, coffee, cucumber, grapefruits, lemon grass, onion, oat, pepper, pomegranate, potato, and rapeseeds), β-sitosterol (widely distributed; main sources: vegetable oils, nuts, and avocados) (**27**), and stigmasterol (main sources: vegetable oils, legumes, nuts, and seeds) (**28**) (Fig. 11) (Sarker and Nahar 2007a; Ogbe et al. 2015).

Phytosterols are structurally similar to cholesterol. When phytosterols are consumed, they can compete with cholesterol for intestinal absorption, resulting in significant reduction in blood cholesterol levels (cholesterol lowering property) in humans (Recette et al. 2010).

In addition, phytosterols possess anti-inflammatory, anti-osteoarthritis, anticancer (against breast, colon, prostate, rectal, and stomach cancers), and immunity-stimulating properties and provide protection against cardiovascular diseases (Trawtwein and Demonty 2007). Phytosterols are also effective in the management of obesity as they can induce weight loss.

1.3.10 Terpenoids

Terpenoids form one of the largest and most diverse groups of phytonutrients. They are found in fruits, vegetables, nuts, and spices (Sarker and Nahar 2007a). To date, there are over 55,000 individual terpenoids reported in the literature. Terpenoids are also known as isoprenoids and derived from a combination of two or more isoprene units (a five-carbon unit, chemically known as 2-methyl-1,3-butadiene).

Terpenoids can be divided into subclasses: monoterpenes (two isoprene units, 10 carbon atoms), sesquiterpenes (3 isoprene units, 15 carbon atoms), diterpenes (4 isoprene units, 20 carbon atoms), triterpenes (6 isoprene units, 30 carbon atoms), tetraterpenes (8 isoprene units, 40 carbon atoms), and polymeric terpenoids (several isoprene units, more than 40 carbon atoms). Some examples of dietary sources of various types of terpenoids are presented in Table 2.

Monoterpenes found in phyto-foods are important as flavoring agents in pharmaceutical, confectionary, and perfume products and possess appetite-enhancing, anti-inflammatory, anti-itching, and bactericidal properties. Many terpenoids from phyto-food have various medicinal values, e.g., anticancer, antidiabetic, and antidiarrheal

Table 2 Examples of some terpenoids phytonutrients and their main sources

Terpenoids	Type	Source	Botanical name	Family
Carvacrol	Monoterpene	Thyme	*Thymus vulgaris*	Lamiaceae
(+)-Limonene		Lemon	*Citrus limon*	Rutaceae
β-Caryophyllene	Sesquiterpene	Cinnamon	*Cinnamomum zeylanicum*	Lauraceae
Curzerenone		Turmeric	*Curcuma longa*	Zingiberaceae
Trans-β-Farnesene		Sweet potato	*Ipomoea batatas*	Convolvulaceae
Retinol	Diterpene	Carrot	*Daucus carota*	Apiaceae
Trans-retinoid acid		Mango	*Mangifera indica*	Anacardiaceae
Cucurbitacin E	Triterpene	Bitter gourd	*Momordica charantia*	Cucurbitaceae
Lupeol		Figs	*Ficus carica*	Moraceae
Capsanthin	Tetraterpene	Capsicum	*Capsicum annum*	Solanaceae

applications, and they are beneficial to human health and well-being (Rabi and Gupta 2008; Khan et al. 2018). It has also been suggested that daily intake of phyto-foods, containing terpenoids, might be useful for the management for obesity-induced metabolic disorders, such as type 2 diabetes, hyperlipidemia, insulin resistance, and cardiovascular diseases (Goto et al. 2010). Some of the subsequent *chapters* will deal with dietary terpenoids of various classes.

1.4 Conclusion

As more and more clinical trials and detailed mechanistic studies have now been carried out to establish the potential health benefits as well as safety margins of different types of phytonutrients present in our diets, the importance of phyto-foods in our diets has become more apparent. Phytonutrients are in no way any replacements for conventional drugs, but evidence suggests that they can certainly contribute to prevention of various chronic and severe illnesses and maintenance regime of our health and well-being through properly planned diets.

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Antioxidants in Diets and Food

2

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Abstract

Nowadays, the society looks with increasing interest in the diet, which is no longer simply a means by which to take the necessary nutrients but has become a means by which it is possible to preserve the health state. German philosopher Ludwig Feuerbach once said: "We are what we eat." This statement is even truer today because of the hectic life, people often tend to eat frugal, fast, and often very fat and unhealthy meals. Today, there is a very high attention both from the social point of view and by the scientific community to the so-called functional foods. These foods, which contain several bioactive compounds, exert innumerable biological properties and therefore can help to preserve the health state. In addition, the use of nutraceuticals has taken the upper hand, unfortunately, often also to the detriment of functional foods, which unlike the former still remain foods and, as such, with a certainly better efficacy-safety profile. The term nutraceutical, indeed, does not refer to a food but rather to a formulation containing one or more compounds isolated from plant extracts or one or more titrated plant extracts. Moreover, some formulations (capsules, tablets, powders, etc.) are rarely studied before being commercialized, and their effectiveness remains rather doubtful in light of a very lax regulation in many countries worldwide.

Keywords

Diet · Antioxidants · Phytochemicals · Extra virgin olive oil · Edible berries · Grapes and wine · Pomegranate · Tree nuts · Brassicaceae vegetables · Herbs and spices

"Let food be thy medicine and medicine be thy food." Hippocrates (400 BC).

2.1 Introduction

Most bioactive food constituents are derived from fruits and vegetables that are rich in minerals and phytochemicals, such as vitamins, carotenoids, phenolic acids, flavonoids, and proanthocyanidins. These substances play an important role as dietary antioxidants, improving cellular defenses, and contributing to counteract the oxidative stress related to aging, as well as to a variety of chronic and degenerative diseases. However, the bioavailability of phytochemical antioxidants can vary according to several factors, such as plant cultivar, growth conditions, harvesting time, and food processing. Moreover, the specific chemical and biological properties of each phytochemical have an impact on absorption and metabolism, thus giving

rise to different effects on the oxidative stress of cellular or tissue compartments (Institute of Medicine of the National Academies 2000; Carlsen et al. 2010). The last release of the USDA National Nutrient Database for Standard Reference (US Department of Agriculture, USDA 2018) has incorporated the vast majority of foods that can be used to develop nutrient values, listed in the Food and Nutrient Database for Dietary Studies (FNDDS). Many studies have been focused on the antioxidant power of most commonly consumed plant-derived foods, showing that very large differences can be found in their antioxidant power, with very high values found for spices, herbs, berries, fruits, nuts, chocolate, and vegetables (Carlsen et al. 2010). Wu et al. (2004) reported highest antioxidant value for cranberries, blueberries, and blackberries among fruits and for beans, artichokes, and russet potatoes among vegetables. Moreover, nuts (pecans, walnuts, and hazelnuts) and spices (ground cloves, ground cinnamon, and oregano) have also been reported as good sources of antioxidants.

This chapter will focus in particular on selected foods that represent the keystone of the Mediterranean diet, antioxidant diet for excellence characterized by a high consumption of plant-based foods, as well as on foods and their isolated bioactive compounds that have become part of our diet as such (functional foods) or as enriched foods, food supplements, nutraceuticals, etc. In particular, it talks about plant foods and their products well-known for nutritional and traditional medical applications, such as olive oil, grapes and wine, edible berries, pomegranate, Brassicaceae, nuts, herbs, and spices (Fig. 1). All these products can be included among the so-called functional foods, used for well-being and able to prevent the onset of chronic diseases, promoting longevity (Motohashi et al. 2017).

The society, indeed, looks with increasing interest to the adoption of a healthier lifestyle and consequently to a diet that cannot only adequately nourish the body but also provide it with all the resources necessary to prevent the onset of oxidative stress-related diseases, today always more diffused.

2.2 Bioactive Constituents

The term “phytochemicals” refers to a plethora of plant-derived compounds that can be divided in two large groups: nutrient and non-nutrient compounds. Carbohydrates, lipids, and proteins as well as some non-essential nutrients such as vitamins and minerals belong to the first group. Secondary metabolites such as alkaloids, terpenes, organic acids, as well as phenols, organosulfurs, iridoids, steroids, saponins, and so on, whose importance is mostly of ecological nature, as they are used as defense mechanisms against predators (herbivorous animals, pathogens, etc.), for interspecific competition or to facilitate reproductive processes (Gul et al. 2016), belong to the second group. In contrast to primary metabolites, these products are not ubiquitous in the living organism and are not expressed continuously; indeed, their concentration and profiles are affected by

Fig. 1 Functional foods well-known for nutritional and traditional medical applications

genotype and pedo-climatic conditions (Smeriglio et al. 2016, 2017, 2018a, b). Today, several databases reporting phytochemical content of foods such as US Department of Agriculture (USDA), Phenol-Explorer, and European Food Information Resource (EuroFIR) are available and freely consultable (www.usda.gov; http://phenol-explorer.eu/; www.eurofir.org).

However, what emerges from the recent literature is the great interest in secondary metabolites and in particular toward polyphenols, compounds that are increasingly studied both in vitro and in vivo, with a wide range of health properties (Fig. 2), often directly related to the particular polyphenol class investigated (Costa et al. 2017). Beyond some ubiquitous polyphenol classes, there are some food-specific, which contribute to conferring specific health properties.

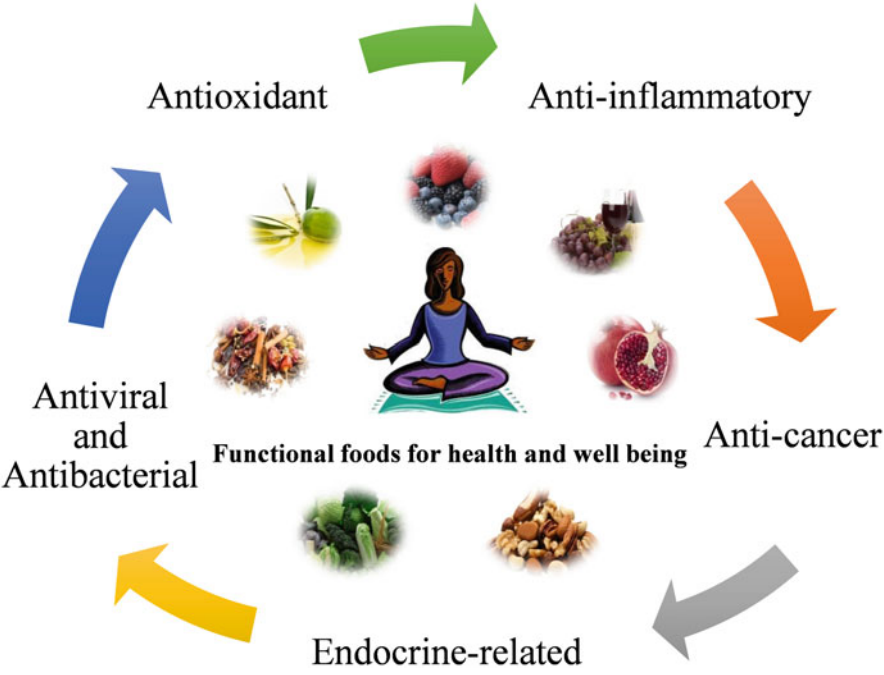


Fig. 2 Health properties of functional foods and their isolated bioactive compounds

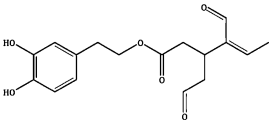
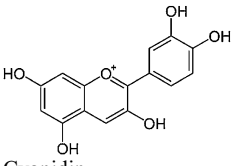
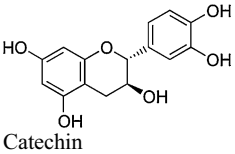
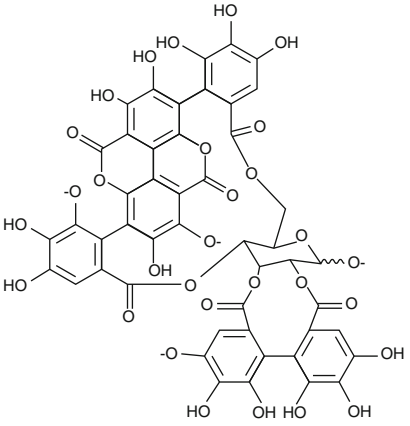
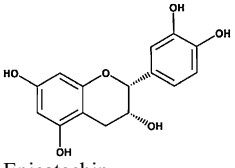
The main polyphenol classes, as well as the chemical structure of most representative compounds identified in most antioxidant functional foods selected, were reported in Table 1 (Serreli and Deiana 2018; Nile and Park 2014; Giovinazzo and Grieco 2015; Wu and Tian 2017; Bolling et al. 2011; Raiola et al. 2017; Guldiken et al. 2018).

2.3 Bioavailability and Metabolism

The study of the pharmacokinetics of polyphenols is fundamental for understanding and evaluating the beneficial properties of these compounds; indeed, to explain their biological activities, polyphenols must be bioavailable, and the dietary abundant ones are not necessarily those that have the best bioavailability profile (D'Archivio et al. 2010).

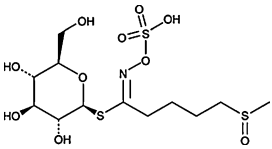
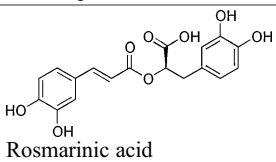
External, food and host organism-related factors affecting the bioavailability of dietary polyphenols in humans. Among the first ones, environmental conditions (i.e., sun exposure, rainfall, degree of ripeness, different type of culture, fruit yield, and so on) and food availability are the main ones. Moreover, several factors are directly related to the food matrix including presence of positive or negative effectors of absorption (i.e., fats, fibers, and proteins), interaction with other polyphenols with

Table 1 Polyphenol compounds in selected functional foods

Functional foods	Main polyphenol classes	Chemical structure of most representative compounds
Extra virgin olive oil 	Secoiridoids, phenylethanoids, phenolic acids, flavonoids, hydroxy-isocromans, lignans	 3,4-DHPEA-EDA
Edible berries 	Anthocyanins, flavonols, catechins, phenolic acids, tannins, stilbenes, lignans	 Cyanidin
Grapes and wine 	Flavonols, flavan-3-ols, anthocyanins, dihydroflavonols, proanthocyanidins, hydroxybenzoic acids, hydroxycinnamates, stilbenoids	 Catechin
Pomegranate 	Ellagitannins, gallotannins, flavonoids, lignans, triterpenoids, phytosterols	 Punicalagin
Nuts 	Flavonoids, phenolic acids, gallotannins, lignans, ellagitannins, stilbenes	 Epicatechin

(continued)

Table 1 (continued)

Functional foods	Main polyphenol classes	Chemical structure of most representative compounds
Brassicaceae 	Glucosinolates, phenolic acids, and flavonoids	 Glucoraphanin
Spices 	Phenolic acids, terpenes, flavonoids, phenylpropanoids	 Rosmarinic acid

similar chemical structure, as well as food concentration and amount introduced. In addition, food processing methods (i.e., thermal treatments, homogenization, lyophilization, cooking methods, and storage) can influence a lot the polyphenols bioavailabily. Finally, other important factors to take into account are related to the host, such as intestinal (i.e., enzyme activity, intestinal transit time, and microbiota) as well as systemic variability (i.e., gender and age, genetics, diseases, and not less important particular physiological conditions) (D'Archivio et al. 2010).

2.3.1 Extra Virgin Olive Oil

Extra virgin olive oil (EVOO) is one of the most investigated foods for its polyphenol profile and pharmacokinetic characteristics. A significant, although very variable, absorption (~40–95%) of the main polyphenols, hydroxytyrosol (HT) and tyrosol (Tyr) (Rodriguez-Morato et al. 2016), as well as other minor compounds such as hydroxycinnamic and hydroxybenzoic acid derivatives (Feliciano et al. 2016; Zhao and Moghadasian 2008) and flavonoids (Ma et al. 2017, 2018), has already been demonstrated. The dietary intake of EVOO polyphenols, in the context of a balanced diet, has been estimated at around 9 mg, of which free HT and Tyr represent at least 1 mg; the remaining part is mainly composed of elenolic esters, oleuropein and ligstroside aglycones (De la Torre 2008; Trombetta et al. 2017). Several in vivo studies have shown how HT and Tyr are absorbed and exert their biological effects in a dose-dependent manner, even at lower doses than the traditional daily dietary intake of Mediterranean countries (Serreli and Deiana 2018). Moreover, about 98% of these polyphenols were found in the plasma and urinary compartment mainly in glucuronidated and sulfated forms (Serreli and Deiana 2018). After ingestion, the EVOO polyphenols may be partially modified in the

gastric environment. The most affected ones are the secoiridoid aglycones. They are normally subjected to a time-dependent hydrolysis in the acid gastric environment, leading to a significant increase in the free HT and Tyr amount already after 30 minutes, while the glycosylated secoiridoids reach the small intestine unmodified (Serreli and Deiana 2018). However, under physiological pH and transit gastric time conditions, some molecules reach the small intestine unmodified. Being particularly concentrated in the intestinal lumen, they might exert a significant local action modulating the oxidative status as well as the inflammation and immune response (Deiana et al. 2018). Following the ingestion of EVOO, the HT and Tyr levels increase rapidly reaching a maximum plasma and urinary concentration after about 1 and 2 hours, respectively, demonstrating how the small intestine is the main absorption site (Serreli and Deiana 2018). The mechanism has not been fully clarified, although it has been demonstrated, by in vitro Caco-2 transwell model that HT transport occurs via a bidirectional passive diffusion (D'Antuono et al. 2016). Moreover, it has been suggested that the different physicochemical properties of EVOO polyphenols may play a pivotal role in the absorption rate; HT and Tyr have the highest bioavailability, followed by verbascoside and luteolin (D'Antuono et al. 2016). However, beyond this aspect, it has been shown that the bioavailability of these compounds in the ingested forms is poor due to intensive metabolism, which occurs at different levels (Rodriguez-Morato et al. 2016; Lopez de las Hazas et al. 2016).

Once absorbed, HT and Tyr are widely distributed throughout the body with a marked organotropism for the skeletal muscle, kidney, liver, lungs, and heart (Serreli and Deiana 2018). A recent study by Peyrol et al. (2018) highlighted the key role of bilitranslocase in the internalization of HT and its glucuronide metabolite in endothelial cells. Furthermore, it has been shown that HT and its metabolites, HT sulfate and HT acetate sulfate (Lopez de las Hazas et al. 2016), as well as Tyr (Angeloni et al. 2017), are able to overcome the blood-brain barrier.

In the liver, the EVOO polyphenols are subjected to an important first-pass metabolism, leading mainly to three types of conjugation, methylation, glucuronidation, and sulfation, by catechol-O-methyltransferase (COMT), uridine-50-diphosphate glucuronosyltransferase (UDPGT), and sulfotransferase (SULT) (Serreli and Deiana 2018).

The sulfated and glucuronidated HT and Tyr are in fact the main metabolites found in human plasma and urine, as well as at the level of the intestinal epithelium, where it is known that they represent the main phase II pathways of the xenobiotic metabolism (Rodriguez-Morato et al. 2016). Another important HT metabolic pathway leads to the homovanillic alcohol formation by COMT (De la Torre et al. 2017). However, it is necessary to underline that HT, homovanillic alcohol, and its derivative homovanillic acid are also endogenously formed by the oxidative metabolism of dopamine, and therefore low concentrations of these metabolites can always be found in biological matrices (Rodriguez-Morato et al. 2016). Glucuronidated metabolites have also been observed for minor components such as luteolin, pinoresinol, and ferulic acid (Silva et al. 2018).

2.3.2 Edible Berries

The chemistry of berry phenols directly influences, other than biological effects, their bioavailability and metabolism. The main structural features of berry phenols regard (1) oxidation degree and hydroxylation patterns, (ii) ability to exist as stereoisomers, (iii) glycosylation, and (iv) polymerization degree (Smeriglio et al. 2016). The main polyphenol classes found in edible berries are anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins, and phenolic acids; however, the most representative one is certainly anthocyanins. These phytochemicals belong to the flavonoid class widely distributed in fruits and vegetables and especially abundant in berries with red, blue, or purple pigments (Nile and Park 2014).

Recent studies indicate that anthocyanins are rapidly absorbed in the stomach by bilitranslocase as well as by the small intestine, in particular at jejunum level, appearing in blood circulation and urine as unmodified, methylated, glucuronide, and/or sulfate conjugated (Passamonti et al. 2003). They appear in the bloodstream within few minutes and reach the maximum plasma concentration after 15–180 min, depending on their chemical features and food matrix (Smeriglio et al. 2016).

Anthocyanins show a marked organotropism for jejunum followed by the stomach, kidney, liver, and brain. Indeed, it has been demonstrated that they can readily cross the blood-brain as well as the blood-retinal barrier. Although their availability to target tissues appears limited, it is consistent to explain their health effects (Smeriglio et al. 2016).

Unlike anthocyanidins, which passively diffuse across the mucosal epithelium, anthocyanins, being highly water-soluble molecules, if not enzymatically hydrolyzed in the small intestine, needed specific carriers to cross the intestine barrier such as sodium-glucose co-transporters (SGLTs) or glucose transporters (GLUTs). However, it has been shown that anthocyanins cross the intestinal barrier preferentially as unmodified molecules (Kamiloglu et al. 2017).

Intestinal microflora rapidly hydrolyze them to the aglycone form, and their bioavailability strictly depends on the sugar moiety; indeed glucose leads to a higher bioavailability than galactose or arabinose (Nile and Park 2014). Derived highly unstable compounds spontaneously degraded into several monomeric phenolic acids and aldehydes such as protocatechuic acid and phloroglucinol aldehyde, which however, contribute to the health effects exerted by native molecules (Smeriglio et al. 2016).

Once in circulation, the metabolites can be subjected to phase II liver metabolism by drug detoxification enzymes such as COMT, SULT1, and UDPGT (Nile and Park 2014).

Many in vivo studies showed that after consumption of a dietary amount of edible berries, no detectable quantities of native anthocyanins or their metabolites were found in plasma, while they were found in the urine in quantities $<0.1\%$ of ingested doses, suggesting poor intestinal absorption (Smeriglio et al. 2016). Several in vivo studies suggest that the food matrix can affect the pharmacokinetics of anthocyanins. From this point of view, phytic acid, contained mainly in the hulls of nuts, seeds, and grain, increases the bioavailability of anthocyanins reducing gastrointestinal

mobility and slowing the passage of anthocyanins through the stomach, duodenum, and jejunum favoring their absorption (Smeriglio et al. 2016).

Another important aspect, which influences anthocyanin bioavailability, is the individual variations in the xenobiotic metabolism due to human polymorphisms, which affects the main enzymes involved in the biotransformation of these bioactive compounds (Smeriglio et al. 2016).

2.3.3 Grape and Wine

Flavonoids and stilbenoids are the representative polyphenol classes in the grape berry skins and consequently in the wine. However, during winemaking, just a small part shifted to the wine, and the final yield strongly depends on the contact rate and grape variety (Giovinazzo and Grieco 2015). In addition to technological factors, which govern the winemaking procedures, several microbiological, chemical, and physical factors contribute to modify the phenol's structure and concentration during the fermentation, fining, and storage of wine (Giovinazzo and Grieco 2015).

Polyphenols are synthetized by grapevine during normal development in response to abiotic and biotic stressors (Degu et al. 2016). Harvest time plays a pivotal role to confer the typical phenol profile to the berries, and the concentration of the different polyphenol classes may change after in relation to postharvest handling protocols (Giovinazzo and Grieco 2015).

Two main polyphenol families can be distinguished in grapevine: flavonoids and non-flavonoids. Flavonoids class includes flavonols, both glycosides and aglycones, flavanols, anthocyanins, dihydroflavonols, and proanthocyanidins (Amor et al. 2018), while the non-flavonoid class includes hydroxybenzoic and hydroxycinnamic acids as well as stilbenoids (Amor et al. 2018).

Unlike what is believed, the predominant polyphenol class in grapevine is that of flavanols with catechin, epicatechin, and proanthocyanidins as the most abundant compounds. They accumulate in the seeds and skin of the grape berries representing the 13–30% of the total phenolic content (Giovinazzo and Grieco 2015). Flavonols are the second most abundant flavonoids, whose profile strongly depends on grapevine varieties. They are present as glycosides in grape skin and as aglycones in wine and juice because, during processing and storage, acid hydrolysis occurs (Giovinazzo and Grieco 2015). Kaempferol, quercetin, and isorhamnetin are the most abundant flavonols in both red and white grapes, whereas myricetin and its derivatives are present only in red grapevine varieties (Giovinazzo and Grieco 2015). Furthermore, recently, acetylated and p-coumaroylated derivatives of isorhamnetin, laricitrin, and syringetin have been identified for the first time in grape skins and wines (Favre et al. 2018).

Anthocyanins can be detected only in red grape varieties, and their biosynthesis is closely related with berry development. Each grape species and variety has a different anthocyanin pattern. They accumulate mainly in the berry skin, although in some cultivars they may accumulate also in the berry flesh (Giovinazzo and Grieco 2015). Anthocyanins and proanthocyanidins are among the most important

compounds in red wine quality because they influence color, bitterness, astringency, and chemical stability to oxidation, the main features of this beverage (Giovinazzo and Grieco 2015).

Stilbenoids are certainly another well-known polyphenol class of grape and wine stilbenoids. Among these, cis−/trans-resveratrol is undoubtedly the leader followed by resveratrol-3-*O*-glucoside (piceid), piceatannol, and viniferins (Giovinazzo and Grieco 2015).

With the exception of anthocyanins, whose absorption and metabolism were reported in Sect. 3.2, most polyphenols cannot be absorbed intact after consumption and have to be modified by the intestinal enzymes or by the colonic microflora. The degree of modification they undergo as well as their bioavailability depends essentially on their chemical structure. The main absorption occurs in the small intestine, where the glycosides are hydrolyzed, while the other polyphenols arrive unchanged in the colon, where they are subjected to extensive structural modifications by colonic microflora, leading to the production of specific active metabolites, i.e., flavonoid aglycones and phenolic acids, which exert biological activities ascribed to the parent molecules. From this point of view, the inter-individual variability plays a pivotal role in producing these active metabolites (Giovinazzo and Grieco 2015).

Once absorbed, polyphenols undergo further structural modifications due to conjugation processes, which mainly include methylation, sulfation, and glucuronidation, which could affect their bioavailability and, consequently, their biological activity (Giovinazzo and Grieco 2015). The polyphenol class which shows the most concerns, from this point of view, is that of stilbenoids.

Indeed, despite various in vitro and in vivo biological activities that have been attributed to stilbenoids, many concerns remain about the translation of results to humans (Dvorakova and Landa 2017). Resveratrol and other resveratrol-like stilbenoids are characterized by a very low bioavailability and extensive metabolism. When administered orally, only about 1–2% of the resveratrol dose can be found in plasma; furthermore, its half-life is 8–14 min due to its rapid metabolism, which occurs predominantly by phase II and, to a lesser extent, by phase I enzymes (Dvorakova and Landa 2017). The first metabolism leads to the corresponding mono-glucuronides or mono-sulfates, while the second one to piceatannol. The most abundant resveratrol metabolites are trans-resveratrol-3-*O*-glucuronide and trans-resveratrol-3-*O*-sulfate, although, depending on the dose administered, the metabolism can shift predominantly toward sulfation. Moreover, the human microbiota metabolizes resveratrol into dihydroresveratrol. Regarding other stilbenoids, informations about metabolism are rather limited (Dvorakova and Landa 2017).

2.3.4 Pomegranate

Numerous polyphenol classes have been identified in pomegranate (Table 1). However, hydrolyzable tannins (HTs) are the main and, among others, the most investigated in this functional food. They can be grouped into ellagitannins (ETs) and gallotannins (GTs) based on the different phenolic acids that are esterified with a

polyol residue (mainly D-glucose). ETs are widespread in nature and can be found in several plant families, while GTs are less common. Pomegranate fruit is rich in ETs. Among them, punicalagin isomers constitute up to 85% (w/w) of total tannins extracted from pomegranate fruit peel (Wu and Tian 2017), although it is also a rich source of ellagic acid and its methylated and glycosidic derivatives (Smeriglio et al. 2017).

On the contrary to simple phenols, whose pharmacokinetics have been extensively investigated by several animal and human studies (Velderrain-Rodríguez et al. 2014), the results about tannin metabolic fate are still scarce and controversial. Furthermore, unlike proanthocyanidins (condensed tannins), few studies on the bioavailability of HTs are currently available.

Recently, Nuñez-Sánchez et al. (2014) investigated the absorption of the main pomegranate juice polyphenols after oral administration. They detected low plasma concentrations of ellagic acid after 0.5–3 h, while no parent compounds (ETs) were detected. This behavior can be ascribed probably to the low water solubility of ellagic acid as well as to its ability to chelate calcium and magnesium ions at intestinal level, leading to nonabsorbable complex (Smeriglio et al. 2017). The main ellagic acid derivatives have been found in the human plasma and urine (Nuñez-Sánchez et al. 2014).

Tannins, because of their complex structure, cannot be absorbed at the gastric level and are partially bioavailable for absorption in the gut tract, particularly in the small intestine for HTs. Furthermore, the gut absorption is strictly related to their polymerization degree (Smeriglio et al. 2017).

Several in vitro studies showed that ETs are stable in the gastric environment, in the presence of gastric and pancreatic enzymes as well as in the presence of bile salts. The best conditions for hydrolysis of ETs are at neutral-alkaline pH (pH 7.1–8.4), typical of the duodenum and the small intestine environment (Smeriglio et al. 2017). During the remaining phases of digestion, the human microbiota metabolizes ETs and ellagic acid to dibenzopyranone metabolites, namely, urolithin A and B. These metabolites are absorbed by the gut in which they undergo glucuronidation. Furthermore, because urolithin A undergoes hydroxylation by cytochrome P450, it may have an improved ability to glucuronidate and enhance the excretion of specific metabolites (Smeriglio et al. 2017).

However, a clinical trial performed on human volunteers fed with a single dose of ellagitannin-rich diet demonstrated, once again, a large inter-individual variability in the pomegranate metabolite profile (Espín et al. 2013).

2.3.5 Tree Nuts

The phytochemical profile of tree nuts varies considerably by nut type, genotype, pre- and postharvest conditions, as well as storage conditions. However, flavonoids are the most abundant polyphenol class with flavan-3-ols, which are the main subclass, followed by flavonols and anthocyanins (Bolling et al. 2011).

Recently, the total phenol value of nine tree nuts was reviewed. This value range from 197 to 1602 mg gallic acid equivalent/100 g with walnuts and pecans, which are the top two, followed by pistachios, hazelnuts, almonds, Brazil nuts, cashews, macadamias, and pine nuts (Bolling et al. 2011). Flavonoids are predominantly present in the food matrix in glycosylated form, which may be partially hydrolyzed by lactase-phlorizin hydrolase (LPH) in the epithelium or in the lumen of the small intestine. In this way, most lipophilic aglycones may enter the epithelial cells by passive diffusion. Alternatively, glycosides can be directly transported in the epithelium via epithelial glucose transporters; subsequently they are hydrolyzed by intracellular β-glycosidase. Unlike dietary nutrients, large amounts of ingested flavonoids are unabsorbed in the proximal intestine and reach the colon where they undergo microbiota-mediated hydrolysis and fermentation (Cassidy and Minihane 2017).

Within the epithelium, flavonoids undergo phase I metabolism with the resultant metabolites transported to the liver via the portal vein. Reached the liver, they undergo further phase I (oxidation or methylation) and phase II metabolisms (glucuronidation, sulfation, or methylation) that result in more polar bioactive compounds that are mainly glucuronidated. The first one occurs by several cytochrome P450 isoforms, in which cytochromes 1A1, 1A2, 1B1, 3A4, and 2C9 are those mainly involved in the flavonoid metabolism, while in the second one, UGTs, sulfotransferases, and COMTs are involved. The latter play a pivotal role in the flavan-3-ol subclass metabolism because they are involved in the *O*-methylation of catecholic flavonoids including catechins, epicatechins, and epigallocatechins (Cassidy and Minihane 2017).

The elimination of flavonoids occurs via urine and bile excretion by which they enter into enterohepatic recycling (Cassidy and Minihane 2017). However, several transporter proteins such as P-glycoprotein are also involved in the flavonoid efflux through the membrane into the portal bloodstream, facilitating absorption, or came back into the intestinal lumen, reducing bioavailability (Cassidy and Minihane 2017).

Recently, another important aspect was highlighted regarding nut polyphenol bioaccessibility: the food matrix effect (Mandalari et al. 2016). The rate and extent of polyphenols released in the gastrointestinal tract, after gastric digestion and gastric plus duodenal digestion, from natural and blanched almond skin (NS and BS, respectively) extract simply diluted in water and milk and incorporated into bakery products, were investigated. Results showed that phenolic acids were the most bioaccessible polyphenols. When diluted in water, NS statistically increased the release of flavan-3-ols and flavonols, whereas bakery products better BS polyphenols. The samples diluted in milk showed the lowest polyphenol recovery and free total phenols content as well as the lowest antioxidant activity, probably due to an interaction between polyphenols and milk proteins, which decreases the bioaccessibility of almond polyphenols. These results could explain the difference observed between polyphenol bioavailability and almond consumption as tale or as part of more complex food matrix (Mandalari et al. 2016).

2.3.6 Brassicaceae Vegetables

Plants belonging to the Brassicaceae family are characterized by a typical polyphenol class, the glucosinolates. These bioactive compounds, relatively stable in the plant cell, contain a thiohydroximate-*O*-sulfonate group linked to glucose and about 200 different alkyl, aralkyl, or indolyl side chains (Barba et al. 2016). When the plant tissue is subjected to cutting, chopping, or mixing, a β-thioglucosidase called myrosinase, normally stored separately, is released, leading to glucosinolate hydrolysis (Oliviero et al. 2018). Glucose and an unstable aglycone, the thiohydroximate-*O*-sulfonate, are the reaction intermediates. They spontaneously reorganize themselves by releasing sulfate ion and several metabolites strictly related to the nature of the side chain of the parent compound and the physicochemical conditions of the medium. For example, acid pH and ferrous iron-rich environment leads to the formation of nitrile derivatives, while neutral pH favors the formation of isothiocyanates (ITCs). ITCs are too unstable compounds, and they easily split into thiocyanate ion and indole-3-carbinol (Capuano et al. 2017).

Normally, dietary intake of Brassicaceae vegetables occurs after cooking, and the breakdown products of glucosinolates is responsible for the typical aroma that is released. Cooking tends to denaturate myrosinase, and this event is strictly dependent on cooking methods used such as boiling, steaming, or microwaving and cooking time. Enzymatic inactivation makes that glucosinolates reach the colon unmodified where they are metabolized by the intestinal microbiota, leading to break down of molecules, which are absorbed and/or excreted.

However, several in vitro and in vivo studies investigated the absorption and metabolic fate of uncooked Brassicaceae vegetables showing as intact glucosinolates could be partially absorbed in the stomach. The unabsorbed part transits through the gastrointestinal tract and as a result of gastric and gastric plus duodenal digestion, which promotes plant myrosinase release, reaches the small intestine where they are hydrolyzed, leading to break down of products, which may be easily absorbed. The remaining non-hydrolyzed glucosinolates reach the colon where they could be hydrolyzed by bacterial myrosinase, leading to break down of molecules, which are absorbed and/or excreted. The presence of bacterial myrosinase activity was corroborated by several studies, which observed the ITC formation in the human feces post-incubation of pure glucosinolates or Brassicaceae vegetable juices in which the enzyme was inactivated by heating (Barba et al. 2016; Capuano et al. 2017; Oliviero et al. 2018).

Since the formation of several breakdown products by intestinal microbiota is very likely, up to date, this aspect is still under investigation, and in humans, only urinary excretion of conjugated metabolites of ITCs, following the consumption of Brassicaceae cooked vegetables, was observed. However, several in vitro investigations showed that secondary degradation of ITCs can lead to the formation of amines, and *Bifidobacterium* strains are able to metabolize glucosinolates to nitriles, which in turn can be converted by other microorganisms into ammonia and organic acids (Barba et al. 2016).

Nevertheless, as seen with other polyphenol classes, the diversity of the enzymatic pattern is associated with the generation of a wider range of metabolites than those so far identified (Oliviero et al. 2018).

Animal studies with radiolabeled ITCs showed a rapid absorption with a peak plasma concentration observed 3 h after ingestion. Absorbed ITCs are then conjugated to glutathione in the liver and excreted in the urine as mercapturic acid (12–80% of the ingested dose), while secondary excretion routes are in the form of CO_2 in the expired air or as unknown metabolites in feces. In humans, the mercapturic acid metabolic pathway is the predominant one, with a good statistical correlation between mercapturic acid excretion and amount of ITCs consumed, so much so that it is considered a biomarker of ITC intake (Oliviero et al. 2018). However, the ITC bioavailability in humans strictly depends on the respective contributions of plant myrosinase and intestinal microbiota in the hydrolysis of glucosinolates, with an increased bioavailability after ingestion of raw Brassicaceae vegetables. Indeed, urine excretion of mercapturic acid after consumption of raw Brassicaceae vegetables, which occurs after 8 h of ingestion, accounts for 17–88% of the ingested dose of glucosinolates and depends on several factors such as molecule type, plant matrix (cabbage, watercress, broccoli, etc.), and human enzymatic pattern. When the vegetables are cooked, this rate does not exceed 20%, and the peak of mercapturic acid in urine occurs only after 12 h of ingestion (Barba et al. 2016).

Glucosinolate metabolites undergo enterohepatic circulation. ITCs showed a pronounced organotropism for the intestinal mucosa, liver, kidneys, bladder, lungs, and spleen, while the body disposition of other glucosinolate breakdown products is still poorly understood. Similar to ITCs, nitriles and epithionitriles could be metabolized and excreted in the urine as mercapturic acid, whereas oxazolidine-2-thione and thiocyanate ion are directly excreted unchanged (Barba et al. 2016; Capuano et al. 2017; Oliviero et al. 2018).

2.3.7 Spices

Besides their use as flavoring, coloring agents, and preservatives, herbs and spices possess several health properties ascribed mainly to the presence of different phytochemicals of which polyphenols represent the class most investigated. Among them, phenolic acids are certainly the predominant ones (Guldiken et al. 2018). However, to understand better their health effects, it is essential to establish the bioavailability of their bioactive constituents.

Phenolic acids, such as hydroxycinnamic and hydroxybenzoic acids, are a class of ubiquitous polyphenols in the plant kingdom. They are present in plant tissues as complexes with lignin and carbohydrates and proteins with which they are able to establish hydrogen bonds by hydroxyl groups in the aromatic ring and ester linkages, respectively. These phenolic complexes are able to resist to gastric and duodenal digestion reaching the colon where they undergo metabolization by microbiota.

Hydroxycinnamic acids are the most diffused and herbs and spices are the richest sources (Mosele et al. 2015).

Caffeic, ferulic, coumaric, as well as chlorogenic and rosmarinic acids (esters of caffeic acid) are the main compounds included in this group. In vitro experiments of simulated microbial metabolism showed that chlorogenic acid undergoes ester hydrolysis, leading to quinic acid and then to hippuric acid and caffeic acid formation, which in turn can be dehydroxylated to coumaric acid and then degraded to hydroxylated phenylpropionic acids or directly dehydrogenated to dihydroxylated phenylpropionic acids. Anyway, mono- and di-dihydroxylated phenylpropionic acids are the main metabolites detected (Mosele et al. 2015). This metabolic pathway was corroborated by detection in vivo of m-coumaric and hippuric acids, after chlorogenic acid administration (Mosele et al. 2015). Rosmarinic acid, being too an ester of caffeic acid, shares the same metabolic pathway with chlorogenic acid once hydrolyzed to caffeic acid.

Ferulic acid, another common hydroxycinnamic acid, has a common metabolic pathway of the previous ones because it can give, by simple dehydrogenation, dihydroferulic acid, which in turn can form, by demethylation, dihydroxyphenylpropionic acid and alternatively, by dehydrogenation followed by α-oxidation, can give vanillic acid (Mosele et al. 2015). However, different profiles of metabolites were observed between in vitro and in vivo studies. This is due both to a large human inter-variability, related to a specific microbiota composition, and to hepatic and renal metabolism, whose metabolites undergo before urine excretion (Mosele et al. 2015).

Hippuric acid was the common phenolic metabolite detected in plasma and urine after the intake of different phenolic acids. Hippuric acid, other than derived by microbial transformation of the quinic acid moiety, as described above, can be derived by the hepatic metabolism of benzoic acids. Nevertheless, the concentration of hippuric acid in urine decreased considerably after the antibiotic treatment and in the urine of ileostomy patients, suggesting an important contribution by the gut microbiota (Mosele et al. 2015). In light of these results, the intestinal absorption of dietary phenolic acids into systemic circulation is poor, although they can exert their activity at the intestinal level (Opara and Chohan 2014; Guldiken et al. 2018).

From this point of view, several in vitro studies on Caco-2 cell transwell model have been very useful to understand the intestinal absorption of phenolic acids from culinary herbs, although they must be correctly interpreted. Indeed, often it happens to compare studies, which use the same experimental model, but starting from samples of very different nature. In particular, the critical points are the starting material (herbs and spices or pure compounds) and amount of starting material, in particular if consistent with a dietary intake and if the starting material was applied as tale or after cooking and/or digestion. In light of these critical points, indeed, very different results can be achieved (Opara and Chohan 2014). However, critically evaluating various in vitro and in vivo studies carried out to date, it can only confirm that phenolic acids have a very low bioavailability and that they carry out most of their effects at the intestinal epithelium level, although the mechanisms have not been still clarified. Furthermore, instability during food processing, distribution, and

storage, as well as the food matrix composition are other key factors which limit the potential health benefits of phenolic acids. Different studies showed interactions between phenolic acids and food matrices, such as milk, olive oil, or sugar, suggesting that variations in phenolic acid absorption also occur due to interactions between polyphenols and other food components, such as proteins, which destabilize these compounds, decreasing their bioaccessibility and subsequently their bioavailability (Nunes et al. 2017).

2.4 Bioactivities and Benefits

The biological properties and health effects of the above functional foods have been widely reviewed. Other than their traditional use, many in vitro and in vivo (animal and human) studies performed suggest that their intake may prevent the onset of several chronic diseases (Rigacci and Stefani 2016; Nile and Park 2014; Giovinazzo and Grieco 2015; Wu and Tian 2017; de Souza et al. 2017; Raiola et al. 2017; Guldiken et al. 2018; Smeriglio et al. 2018a). The recognized biological activities have been ascribed predominantly to the polyphenol classes discussed in Sect. 3, although synergistic effects could not be excluded, so much so that often the food as it is, or its raw extract, shows a greater activity than their bioactive isolated compounds.

In addition to their well-documented free radical scavenging and antioxidant activity, these functional foods seem to exert several bioactivities such as antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and endocrine-related activities (Fig. 2).

2.4.1 Extra Virgin Olive Oil

Cultivation of olive tree (Fig. 1e) and olive production date back to about 7000 years ago, while nutritional and medicinal properties of olive oil have been known since the ancient times (Hashmi et al. 2015). EVOO is the most important component of the Mediterranean diet, being its health effects mainly due to a high content in monounsaturated fatty acids and to the presence of phenolic compounds. EVOO nutraceutical properties, as well as biological properties of its minor unsaponifiable fraction components (vitamin E, carotenoids, and phenolic compounds), have been investigated by in vitro and in vivo studies, revealing a protective role in oxidative stress-related diseases (Sabatini et al. 2018; Yubero-Serrano et al. 2019).

The bioavailability of EVOO polyphenol metabolites is often higher than that reached by the ingested parent compounds; therefore, these metabolites are likely to contribute significantly to the health effect correlated to the regular consumption of EVOO. However, although in the last decade several studies have been carried out in order to evaluate the potential health benefits of EVOO phenol metabolites, the data available are still poor. In vitro studies showed that EVOO phenol metabolites are able to exert significant antioxidant and free radical scavenging activity by

modulating several molecular pathways. They perform their activity mainly at the cardiovascular and gastrointestinal level ameliorating physiological condition and preventing exacerbation of inflammation and oxidative stress, sometimes even with greater efficiency than native compounds (Serreli and Deiana 2018).

Different parts of *O. europaea* L. have been widely used in traditional medicine for a wide range of ailments, either alone or in combination with other herbs (Hashmi et al. 2015) showing a broad spectrum of health-promoting properties such as antioxidant, anti-inflammatory, anti-allergic, anti-atherogenic, antithrombotic, and antimutagenic effects (Abdelhalim et al. 2017; Gorzynik-Debicka et al. 2018). In Mediterranean folk medicine, *O. europaea* was also used as a common remedy for gout, for bacterial infections such as gingivitis and otitis, and for icterus, cough, and hair loss as well as febrifuge (Hashmi et al. 2015). Furthermore, it was used also as liver depurative and hypotensive (Cornara et al. 2014).

Traditionally, for its emollient properties and its affinity with the skin, EVOO was also used as a vehicle for drugs or medicinal plants with topical application, whose active ingredients were characterized by lipophilic properties (Leporatti and Ghedira 2009). Lately, this aspect has aroused the interest of researchers, and some studies have been made on the use of nutraceutical oral and topical preparations of hydroxytyrosol and vitamin E conveyed in EVOO. The first important result is the greater bioavailability found for hydroxytyrosol, which, until then, had been always conveyed in water thanks to its amphiphilic properties. The second one is that the presence of other polyphenols naturally present in EVOO seems to synergize the action of hydroxytyrosol and vitamin E, expressing interesting health effects on in vitro cell-based model of skin inflammation (Smeriglio et al. 2019a) as well as on healthy patients predisposed to the onset of oxidative stress-related metabolic diseases (Colica et al. 2017) and even more on patients affected by nonalcoholic fatty liver disease (Nobili et al. 2019).

2.4.2 Edible Berries

Common edible berries include strawberries (genus *Fragaria*), raspberries (Fig. 1a), and blackberries (genus *Rubus*) belonging to the Rosaceae family, blueberries (*Vaccinium myrtillus* L.) and cranberry (*V. macrocarpon* Ait.) belonging to the Ericaceae family, and red currants (*Ribes rubrum* L.) and black currants (*R. nigrum* L.) belonging to the Grossulariaceae family. These small fruits are important commercial food due to their delicious taste and flavor and their healthy properties. Berries are appreciated worldwide both fresh and as transformation products such as juices, jams, tea, desserts, jellies, and bakery products (Monforte et al. 2018). They are mainly rich in phenolic compounds (phenolic acids, flavonoids) and ascorbic acid, both with high antioxidant properties (Skrovankova et al. 2015). Blue, purple, and red colors of berries are due to the presence of anthocyanins, which show a wide variety of health-promoting properties for human health, such as antioxidant, anti-inflammatory, and anti-carcinogenic effects, thereby reducing the incidence of chronic and degenerative diseases (Smeriglio et al. 2016). Many different berries are used worldwide for

nutritional purposes, but also in folk medicine. Recently, the folk medicinal use of different *Rubus* species was reviewed (Sultana 2018). Tea made from the leaves of *R. idaeus* L. was reported as an efficient remedy for wounds, diarrhea, and colic pain or as a uterine relaxant. In addition, the author also refers the use of leaves from other berries, such as blackberry, as an anti-inflammatory agent and raspberry as antibacterial, anti-inflammatory, diaphoretic, diuretic, and choleretic agent (Sultana 2018). A review concerning the ethnobotanical uses of the genus *Vaccinium* (Abreu et al. 2014) has reported many ethnomedical and food uses in 36 *Vaccinium* species, mainly from North America, Asia, and Europe. These medicinal remedies are mainly referred to digestive, genitourinary, and endocrine/metabolic systems, with *V. vitis-idaea* L., *V. myrtillus* L., *V. macrocarpon* Aiton, and *V. uliginosum* L. being the most cited species. A recent study on the ethnobotany of Patagonia (Chamorro et al. 2019) has reported the medicinal use of other berries, described in the Mapuche medical system, e.g., *Berberis microphylla* G.Forst. (Berberidaceae), *Ribes magellanicum* Poir. (Grossulariaceae), and *Luma apiculata* (DC.) Burret (Chilean myrtle, Myrtaceae). Several in vitro and in vivo studies showed that extracts of several cultivars of blackberries, black and red currants, blueberries, and black and red raspberries showed a remarkably scavenging activity toward chemically generated superoxide radicals; they are therefore useful in fighting aging and related diseases. Behavioral studies in rodents showed an attenuation of brain aging after ingestion of strawberries, blueberries, or blackberries. Moreover, berry polyphenols reduce the risk of cardiovascular disease and type 2 diabetes (Nile and Park 2014).

There is a wide literature about anticancer effects of berry polyphenols. Furthermore, several berry-isolated compounds were found to possess antimutagenic activity; among them, the most investigated are ellagitannins and in particular ellagic acid (Nile and Park 2014).

On the contrary, proanthocyanidins, and in particular the A-type of cranberry, may enhance both in vitro and in vivo urinary bacterial anti-adhesion activities. Among the berries, cranberries, cloudberries, red raspberries, strawberries, and bilberries possess clear antimicrobial effects against human pathogens, inhibiting the growth primarily of gram-negative bacteria without any effect on gram-positive bacteria. Berry polyphenols also have vasoprotective and anti-inflammatory activity; indeed several berry anthocyanin-rich extracts are available as food supplement to treat microcirculation disease and to maintain normal vascular permeability. Recently, some in vitro studies have also found black raspberry extracts to be effective in reducing vascular endothelial growth factor expression, a promoter of angiogenesis, which is a critical step for tumor progression and metastasis (Nile and Park 2014).

2.4.3 Grape and Wine

Vitis vinifera L. is a perennial woody vine (Fig. 1c) native to the Mediterranean region, Central Europe, and southwestern Asia. Grape and red wines have been recognized and consumed worldwide for over 2,000 years. One of the earliest

sources that document grape uses is the preparation of wine depicted on the walls of the Theban 18th dynasty tomb chapel of Nakht. The beneficial health effects of wine have been linked to the "French paradox," i.e., the lower incidence of coronary heart disease in the Bordeaux area in France, with respect to northern European countries, despite a similar diet rich in saturated fats. This fact has been related to the traditional consumption in the French region of red wine rich in flavonoids. The latter, indeed, is known for having potent antioxidants with cardioprotective, anti-inflammatory, anticancer, and antimicrobial properties (Georgiev et al. 2014). However, it is good to underline that different grape cultivars show typical flavonoid patterns and have different health-promoting effects on the human body. Giovinazzo and Grieco (2015) have reviewed, recently, the major health effects of grape and wine, ascribing their main biological properties not only to flavonoids but also to the stilbenoid content and in particular to *trans*-resveratrol. Moreover, another important polyphenol class is that of proanthocyanidins, which showed 50 times greater antioxidant activity than vitamin C and 20 times greater antioxidant activity than vitamin E.

The antioxidant properties of grape compounds reduce the risk of atherosclerosis; play a key role in the prevention of heart disease, diabetic neuropathy, and Alzheimer's disease; and exert an immune-modulatory effect. Anthocyanin and *trans*-resveratrol seem able to prevent the onset of cardiovascular diseases and age-related disorders correlated with metabolic syndrome. In addition, the positive effects described in mice subjected to a high-fat diet and supplemented with *trans*-resveratrol open up new approaches for treating also obesity-related disorders. Increased consumption of grapes has also been associated with a reduced risk of cancer. This observation well correlates with the flavonol and *trans*-resveratrol intake (Giovinazzo and Grieco 2015). Another polyphenol, which attracted much attention, was the flavan-3-ol epicatechin, contained in red wine in concentrations higher than other flavonoids. Moreover, winemaking by-products, such as red grape pomace and grape seeds, are also suitable raw materials to obtain antioxidant and anti-inflammatory dietary supplements due to their phytochemical contents such as gallic acid, catechin, and epicatechin, which show an immune-modulatory role in inflammatory conditions (Giovinazzo and Grieco 2015). The polyphenol amount in grape seeds is approximately 60–70%, and in the United States, many commercial preparations of grape seed extract (GSE) with up to 95% procyanidins are marketed as dietary supplement, being appreciated for their powerful protective properties against free radicals and oxidative stress (Smeriglio et al. 2018). Different parts of *V. vinifera* (mainly fruits) have been used also to prepare remedies of traditional medicine (Bombardelli and Morazzonni 1995), e.g., the use of leaves in infusion as hemostatic and for treatment of diarrhea, hepatitis, and stomachaches (Mansour et al. 2013).

2.4.4 Pomegranate

Initially named *Malum punicum*, meaning the apple of Carthage, this specie was then called by Linnaeus *Punica granatum* (Fig. 1g). The common name of the species comes from the Latin words *pomum*, apple, and *granatum*, seeded. The plant has

been cultivated and used for several millennia throughout the Middle East, Southern Asia, and Mediterranean region, while nowadays it is extensively cultivated in Southeast Asia and China. Various cultivars are appreciated for their taste, and "Wonderful" is the most famous commercial variety worldwide. Fruit seeds are consumed fresh, used as spice, and utilized for different culinary purposes. They contain dietary fibers, vitamins, and healthy substances, such as punicalagins and punicic acid, responsible for most of the plant's biological properties. The juice is rich in anthocyanins, in particular pelargonidin glycosides, and other polyphenol classes including ellagitannins (Khan et al. 2017), although the last ones are particularly abundant in the peel and inner membranes of pomegranate. Phenolic acids are present in both fruit and juice, showing significant antioxidant activity and healthy properties (Wu and Tian 2017), recognizing pomegranate as one of the new superfoods.

Many studies and reviews on the pharmacological properties of pomegranate have shown the therapeutic utility of various parts of this plant. The most popular use of pomegranate worldwide is as a vermifugal agent so much so that this fruit is already mentioned in the Eber's papyrus (1550 BCE), testifying that ancient Egyptians used the root extracts of pomegranate in removing intestinal tapeworms (Arun and Singh 2012).

In folk medicine, different parts of the plant have been used, including its flowers, leaves, bark, roots, fruit peel, and juice. In particular, the fruit shows strong astringent effects due to an abundant content of tannins, and therefore, its decoction has been indicated for treating stomach disorders and dysentery. Moreover, the flower infusion has been used to treat diarrhea and infections that cause vaginal discharge. Together with pomegranate peel, the flower infusion has been used to relieve inflammation of the pancreas, while the fruit juice can heal gallbladder diseases. Fresh or dried root barks, or ethanol extracts thereof, have been widely used to remove intestinal parasites. Finally, pomegranate has been used in folk medicine for its antibacterial, anti-inflammatory, antispasmodic, diuretic, carminative, sudorific, galactogogue, and emmenagogue properties (Shaygannia et al. 2016).

Recent studies show that extracts of all parts of the fruit have therapeutic properties and can be useful for a range of diseases, such as diabetes, male infertility, cardiovascular disorders, cancer, and Alzheimer's disease (Sreekumar et al. 2014).

For example, quercetin and ellagic acid, both present in pomegranate, show together a higher inhibitory effect against cancer cell growth. Pomegranate extract has also antiestrogenic effect in the mammary gland, without any adverse effects on cardiovascular and skeletal system; it could be considered a selective estrogen receptor modulator, and further studies are necessary to test its efficacy on estrogen-dependent breast cancers (Sreekumar et al. 2014).

2.4.5 Tree Nuts

Tree nuts are important food sources since prehistoric times, providing beneficial effects to human health due to the presence of protein, fibers, unsaturated fatty acids, minerals (magnesium, potassium, and copper), vitamins (vitamin E, vitamin B6,

folic acid, and niacin), phytosterols, and polyphenols, such as catechins and resveratrol (Vadivel et al. 2012). Several tree nuts, such as walnuts, pecans, almonds (Fig. 1d), and chestnuts, are among the dietary plants with the highest content in antioxidants. Even though limited, the current evidence indicate that tree nut phytochemicals are bioaccessible and bioavailable in humans. Their consumption is associated with a wide range of biological activities including antioxidant, anti-inflammatory, antiproliferative, antiviral, chemopreventive, and hypocholesterolemic actions, decreasing the onset of chronic degenerative diseases (Bolling et al. 2011). In addition, nut intake can be associated with healthy body weight maintenance, because nut fat content consists mainly of unsaturated fats, which able to induce energy expenditure by thermogenesis (Vadivel et al. 2012). Traditional Mediterranean nuts include almonds, hazelnuts, walnuts, pine nuts, and pistachios. Among these, walnuts (*Juglans regia* L.) are most commonly found in our diet and contain different compounds with antioxidant and anti-inflammatory bioactivity. These phytochemicals are responsible for a wide range of health-promoting effects, including lowering of cholesterol, increasing the ratio of high-density lipoprotein cholesterol to total cholesterol, and improving arterial function (Fatima et al. 2018). For these reasons, tree nuts showed promising results on cardiovascular diseases, both in prevention and in the treatment of related diseases. On the contrary, studies, which correlate the tree nut consumption with a decrease of incidence of cancer or metabolic disorders, are still scarce. Another aspect to underline is the allergenic potential of nuts, especially among children. However, the roasted tree nuts are certainly more digestible and less allergenic than natural ones. Consumption of tree nuts, despite being high-energy foods, does not increase weight gain, but it gives satiety and increases thermogenesis as long as a small quantity is consumed within a balanced diet (de Souza et al. 2017).

2.4.6 Brassicaceae

The Brassicaceae family is a vast complex of plant species including different domesticated entities (Fig. 1b). The latter belongs mainly to the genus *Brassica*, but species of the genera *Crambe*, *Eruca*, *Diplotaxis*, *Raphanus*, and *Sinapis* are also included. Domesticated Brassicaceae are among the most important plant foods. They are cultivated as vegetable crop to produce fresh or conserved food, but several ones are also exploited for oilseeds, yielding edible oils (Mcvetty and Duncan 2016). The consumption of Brassicaceae can produce beneficial effects to human health due to the presence of different bioactive compounds. More specifically, the antioxidant activity derives from the presence of well-known bioactive agents in fresh vegetables such as vitamins C and E, carotenoids, and antioxidant enzymes such as catalase, superoxide dismutase, and peroxidase. In addition, Brassicaceae are also rich in flavonoids, coumarins, terpenes, and sulfur-containing compounds such as glucosinolates and S-methylcysteine sulfoxide (Raiola et al. 2017). These compounds have been shown to reduce oxidative stress, especially by inducing detoxification enzymes, and in addition to stimulate the immune system and to reduce the

incidence of cancer (Herr and Büchler 2010). Health-promoting effects of Brassicaceae have been attributed mainly to glucosinolates and in particular to their hydrolyzation products isothiocyanates. Glucosinolates are typical of pungent Brassicaceae such as horseradish, mustard, and cabbage. Isothiocyanates have attracted much interest from a medical point of view due to their anti-inflammatory and anticancer potentials. Several in vitro and in vivo studies suggest a chemopreventive activity of these bioactive compounds through the redox-sensitive transcription factor Nrf2. Furthermore, studies in cultured cells, on animal models, and in humans support the anti-inflammatory effect of *Brassica*-derived phytochemicals. However, the molecular mechanisms by which these bioactive compounds exert their health-promoting properties are still scarce and controversial. Recent findings suggest the involvement of epigenetic mechanisms, being the isothiocyanates able to inhibit histone deacetylase transferases and DNA methyltransferases in cultured cells, but further studies are necessary to corroborate this assumption (Wagner et al. 2013).

The ancient use of Brassicaceae, not only as food but also for medicinal purposes, is confirmed by the mention of *rhaphanos*, or *krambē*, as a remedy against drunkenness and subsequent headache. These news date back to Theophrastus (fourth century B.C.E.), while Dioscorides also cited the plant in his famous book *De Materia Medica* written about the year 65 C.E. (Maggioni et al. 2018).

Recently, different folk medicinal uses of Brassicaceae have been reported, especially concerning the seeds of *Brassica nigra* (L.) W.D.J. Koch, a species cultivated worldwide. The seeds are employed in the treatment of rheumatism and joint pains, indurations of the liver and spleen, and throat tumors and as laxative. Seeds are also appreciated for their antioxidant and antimicrobial activities (Obi et al. 2009). Recently, the leaves of *B. nigra* have been shown to possess therapeutic value too, including hepatic and nephroprotective effects (Rajamurugan et al. 2012).

Due to its antioxidant and anti-inflammatory properties (Rokayya et al. 2014), *Brassica oleracea* L. (cabbage) has been widely used as a traditional remedy for gastrointestinal disorders such as gastritis, peptic and duodenal ulcers, and irritable bowel syndrome. In addition, recent studies have shown that the cabbage can be also used for the treatment of skin inflammation (Lee et al. 2018).

2.4.7 Spices

Spices are different parts of a plant generally used in diet for their aromatic properties but characterized by a low nutritional value. However, these aromatic species contain different phytochemicals, mainly phenolic acids and flavonoids, with powerful antioxidant activity that may still be an important contributor to the antioxidant intake of human diet. A positive correlation was found between total phenol content and antioxidant activity of spices (Guldiken et al. 2018). Such compounds have been selected, through plant evolution, as protection against plant cell oxidative damage (Paur et al. 2011).

A recent study on the antioxidant properties of 425 spices and herbs from 59 different manufacturers or countries showed that clove, in the form of dried sample, had the highest mean antioxidant value, followed by peppermint, allspice, cinnamon, oregano, thyme, sage, rosemary (Fig. 1f), saffron, and estragon. Moreover, fresh samples often showed lower values in comparison with dried material, as confirmed by data obtained for oregano, rosemary, thyme, basil, chives, dill, and parsley (Guldiken et al. 2018). This kind of evidence seems related to the drying process, which preserves most of the phytochemicals intact in the dried products, which, based on the same weight, shows therefore a greater antioxidant activity. Some authors showed that among commercially available spices, the highest antioxidant capacity was found for clove, oregano, and thyme, probably due to the presence of rosmarinic acid in thyme and oregano, eugenol in clove and allspice, and gallic acid in clove. The properties of these compounds could be related to the inhibition of NF-κB transcription factor, involved in immune and inflammatory responses (Paur et al. 2011).

From ancient times, spices were used as food and to prevent and treat chronic health problems, but also as coloring agents, preservatives, and food additives. Different ethnobotanical studies documented the use of spices in traditional medical systems in different countries. Herbs and spices are common to the traditional Mediterranean diet and are widely used in China and India, where the history of spices is more than 7500 years old. The most common spices reported different therapeutic activities, such as purgative, laxative, expectorant, carminative, and diuretic and showed that spices can have synergistic actions with holistic effects on human health (Sachan et al. 2018).

2.5 Application in Food

Bioavailability of phytochemicals, other than influenced by external factors, physicochemical features of bioactive compounds, and host-related factors, is strongly influenced by several food-related factors. The food matrix and food processing are the main key factors involved.

It is well-known that the best way to take a food is to eat it raw (uncooked) to keep all its native nutritional and health properties. However, sometimes this is not feasible or otherwise not preferable.

Thermal treatment affects the content and, consequently, the amount of the bioavailable phenolic compounds. However, it does not influence always negatively the bioavailability of bioactive molecules. Indeed, often, heat treatment leads to the formation of metabolites that show a greater activity compared to native molecules; therefore, sometimes the final biological properties of the food are not affected or, sometimes, may be enhanced, as was observed after thermal treatment of several spices. On the contrary, when the health effects of a given food are ascribable only, or largely, to the native molecules, the cooking, or in any case the food processing in general, significantly affects its biological properties. An example is the EVOO, in which the concentration of hydroxytyrosol, elenolic acid, and oleuropein aglycone

as well as its derivatives decreases fast enough after thermal treatment (D'Archivio et al. 2010). However, not all cooking methods are the same, because the temperatures used differ, in some cases, substantially. Generally, boiling and frying determined a higher loss of total phenols than steaming, although for each vegetable, a preferential cooking method could be selected to preserve or improve its nutritional properties. The steam-cooked broccoli, for example, results in an increase of the polyphenol content and consequently in its antioxidant activity. Another aspect which affects the polyphenol content is the storage of not only fresh fruits and vegetables but also of processed foods such as juices, frozen foods, wine, and so on. After cold storage, broccoli, for example, lost about 75% of its caffeoyl-quinic derivatives and 40–50% of its sinapic acid and feruloyl derivatives (Raiola et al. 2017). In the same manner, an important decrease of phenolic acids was observed in frozen red raspberries. Regarding storage, the light, for some foods in particular, plays a pivotal role; an example is the EVOO. Exposed to light, EVOO lost about 45% of its total phenol content in 4 months. On the contrary, if stored adequately in closed bottles in the dark, the antioxidant activity of EVOO remains unchanged until 8 months. This does not mean that its polyphenol profile does not change but that the hydrolysis of the more complex polyphenols leads to an increase in simpler polyphenol concentration such as hydroxytyrosol and tyrosol, preserving the food's total antioxidant capacity (D'Archivio et al. 2010; Smeriglio et al. 2019b).

Technological processes, such as chopping, milling, and homogenization, could increase the bioavailability of polyphenols by the alteration of the food matrix. It is well-known, indeed, that a direct interaction between polyphenols and some food components, such as proteins, carbohydrates, fiber, and fat, exist, although, sometimes, with controversial results and strictly dependent on the polyphenol class investigated (D'Archivio et al. 2010; Mandalari et al. 2016). Generally, dietary fiber slows the gastric transit, increasing the absorption of the polyphenols, while the fats sometimes sequester the related molecules decreasing their absorption. However, there are exceptions and one of these is the EVOO. Hydroxytyrosol and tyrosol showed an increased bioavailability when administered conveyed in EVOO compared to an aqueous solution, despite their amphiphilic nature (Colica et al. 2017; Nobili et al. 2019).

Another factor affecting the polyphenol bioavailability is the interaction with other compounds. It has been reported that intermolecular bonds between transport protein such as serum albumin and quercetin metabolites can occur, slowing the metabolite elimination. Similarly, epigallocatechin-3-*O*-gallate possesses a high affinity for blood proteins, and this could increase its plasma half-life (D'Archivio et al. 2010).

2.6 Safety: Toxicity and Side Effects

Discussing about food and risk-benefit profile is almost a foregone conclusion if we exclude everything outside the food per se, such as pesticides and heavy metals, and anything else that comes from human intervention and environmental factors. This is

because substantially, it concerns something that has been assumed for decades by humans without showing any undesired effects and because, as seen previously, they contain several bioactive molecules but with low concentrations and often poor bioavailability. However, it is important to make a distinction between foods and food-isolated compounds, which generally can be found in dietary supplements. Food supplements, in fact, contain high amounts of one or more bioactive molecules, which contribute to overall dietary intake, and the potential risks associated with a high daily intake have not been, up to date, deeply investigated. In particular, the most worrisome aspects concern the increased bioavailability of these bioactive compounds that can determine side effects due to potential pharmacokinetic interactions with both endogenous molecules and with other xenobiotic such as drugs (Smeriglio et al. 2017).

Furthermore, other than phytochemicals which showed a wide range and less specific health properties, there are others with a specific activity that can represent a serious risk for human health if not taken in the correct doses and under certain conditions. Among them, those which attracted the most attention lately are the isoflavones. They are able to interfere with the endocrine system causing positive or negative biological effects on organism, its progeny, or subpopulations. Therefore, the main concern regards the potential adverse effects of isoflavones on human health reproduction. Animal and human studies showed, indeed, side effects on female and male reproductive systems upon in utero exposure to isoflavones, in particular to genistein. However, the European Food Safety Authority (EFSA) concluded, based on actual evidences, that in postmenopausal women, isoflavones do not adversely affect the breast, thyroid, and uterus (Smeriglio et al. 2018b).

Another aspect, which is of particular concern, is the maternal ingestion of polyphenol-rich foods as well as dietary supplements, especially during the third trimester of pregnancy. A direct correlation was highlighted between polyphenol-rich diet and fetal ductal constriction, with statistically significant differences between fetuses exposed to high-polyphenol diet and low-polyphenol diet. In particular, the first group showed an increase of ductal velocities, pulsatility index, and right ventricle dimension than those exposed to minimal amounts of these substances. The molecular mechanisms involved have not been fully elucidated. In light of these observations, however, the intake during late pregnancy is not recommended (Hahn et al. 2017).

Nevertheless, also among foods, there is one, which draws particular attention.

Tree nuts have become an important part of human diet worldwide, thanks to several in vitro, animal, and human studies which highlight their ability to control weight and their high-diet values. However, despite the widely accepted health benefits, they are one of the eight foods, such as wheat, milk, egg, soy, fish, and shellfish, responsible for 90% of IgE-mediated allergic reactions in the world. Moreover, it has been observed that, even though the proportion of younger children suffering from tree nut allergies is lower compared to other big eight food allergies, the allergic reactions to tree nuts are more dangerous than other ones, causing most frequent cases of fatalities (Vanga and Raghavan 2017).

Finally, another category of foods most investigated from the safety point of view is spices.

Paracelsus said: "the dose makes the poison"; thus, depending on the dose of exposure, the potential health benefits of spice phytochemicals may become toxic effects including carcinogenic, neurotoxic, genotoxic, teratogenic, cytotoxic, nephrotoxic, hepatotoxic, and gastrointestinal side effects. Among spices, those that are of particular concern are red chili and some commonly used spices such as cinnamon, nutmeg, black pepper, and basil (Guldiken et al. 2018).

A clinical study showed that high chili pepper consumption increases, in a dose-dependent manner, the risk of aerodigestive track cancers of different ethnic-cultural groups, with a strong correlation between the consumption of capsaicin pepper and stomach cancer (Guldiken et al. 2018).

The risk associated instead to commonly spices is strictly correlated to their safrole content. This molecule is able to interfere with DNA, leading to the formation of adducts, and is well-known as a weak hepatocarcinogen. Other spices such as red pepper, fennel, cardamom, dark pepper, cumin, and coriander may activate acid secretion and exert harmful effects on the gastric mucosa.

Furthermore, several adverse effects on human health were observed following co-administration of spices and drugs (Guldiken et al. 2018).

In conclusion, not everything that is natural can be considered safe, and dose-related side or toxic effects should be considered and evaluated. Furthermore, further studies on the potential adverse events that might be associated with high intake of food-isolated bioactive constituents are needed.

2.7 Marketed Products

Today, there is a great availability of nutraceutical formulation such as tablets, powders, capsules, and so on, containing food bioactive compounds. The basic research supports the biological properties ascribed to them, but the health effects on humans are sometimes scarce or controversial. This is due, mainly, to the poor availability of clinical trials and to the difficulty to translate in vitro and animal studies to humans. The main issues are bioavailability, metabolism, dose/response ratio, and side effects of these food bioactive compounds or nutraceutical formulations. Furthermore, in some countries, in which the regulations on nutraceuticals are quite lax, other aspects such as phytochemical characterization, extract standardization and stability of pure compounds, and food extracts and formulation as tale are rather lacking, making sure that there are substantial differences between a product and another product with the same bioactive compounds and between lots of the same product. Apart from these latter ones, which are easily resolvable problems, simply by applying good laboratory practices and conceiving a product seriously from the qualitative point of view, the most problematic aspect remains the bioavailability of many bioactive compounds.

Several technological strategies are to date available to preserve the bioactive compounds by degradation during processing and to improve their bioavailability

and consequently their health effects. The most common procedure, already used for a long time for drugs, is to encapsulate the bioactive compounds in a coating material, which can release its contents at a controlled rate and under specific conditions.

This represents a great challenge, especially for molecules such as polyphenols characterized by insufficient gastric permanence, low intestinal absorption and/or solubility, gastrointestinal tract degradation (i.e., pH, enzymes) and/or interaction (presence of other nutrients, food matrix), as well as chemical instability under industrial processing conditions and storage (i.e., temperature, light, oxygen). The nutraceutical use of these compounds, therefore, requires an adequate formulation that guarantees the native characteristics of the bioactive compounds up to the time of consumption and that allows the active ingredients to reach the target site in order to exert their biological activity.

Several microencapsulation techniques of bioactive compounds are available nowadays, being very widespread in the food and cosmetic industry. They can be mainly divided into physical, physicochemical, chemical, and other stabilization methods. Among these, the most used are the spray-drying, freeze-drying, coacervation, molecular liposome inclusion, co-crystallization, nanoencapsulation, yeast encapsulation, and emulsion (Smeriglio et al. 2014).

2.8 Patents

Several patents are today available about food polyphenols, many of which concern extraction methods, enriched foods, cosmetic formulations, and recently, many nutraceutical formulations.

Remaining consistent with what was the guideline of this chapter, Table 2 shows the international patents related to selected functional foods (extra virgin olive oil, edible berries, grapes and wine, pomegranate, nuts, Brassicaceae, and spices).

In addition to the international patents listed in Table 2, it is worth pointing out that there are also patents in which the foods treated in this chapter are used as a vehicle to convey synthetic active ingredients or plant extract-isolated bioactive compounds. Italian national patent for utility model N°202 017 000 075 448 and Italian national patent for industrial invention N°102 016 000 007 757 are two examples being related to gastric-resistant food/pharmaceutical capsule for the containment and administration of hydroxytyrosol conveyed in EVOO.

2.9 Perspectives

Despite the high number of in vitro and in vivo studies about polyphenols and health properties, the available clinical studies are scarce, controversial, and sometimes with conflicting results because different formulations, dosages, and treatment

Table 2 Patents released in the last 20 years about functional foods selected, based on innovative extraction procedures, formulations, and preservations of active ingredients. (Source: https://worldwide.espacenet.com (only the first 500 results are displayed). Last accessed: March 1, 2019)

Functional food	Patent name	Classification	Application number
Extra virgin olive oil	A novel composition obtainable from extra virgin olive oil and methods of preparation thereof;	International	GB20170009211 20170322
	Reactor for increasing the quantity of polyphenols and/or the turbidity stability of extra-virgin olive oil, system and method using said reactor	International	EP20140731366 20140508
	Method of extracting phenolic fractions of extra virgin olive oil	International	US201214002920 20120302
	Method for the preparation of herbs-enriched extra virgin olive oil-based products	International	GR20110100490 20110812
	Soft capsule for directly drinking extra-virgin olive oil	International	JP20050116225 20050317
	Dietary extra-virgin olive oil with omega-3 fatty acids and relevant production technique	International	WO2003IT00391 20030624
	Process to acquire extra virgin olive oil enriched with vitamin E	International	ES20000000744 20000327
	Process to acquire extra virgin olive oil enriched with vitamins	International	ES20000000743 20000327
Edible berries	Preserving miracle fruit berries	International	US201615227389 20160803
	Sustained-release preservative and preservation method for berries	International	CN201710573890 20170714
	Method for vacuum drying of fruits and berries	International	RU20160132597 20160808
	Method for organic manufacture and increase in duration of garden strawberry berries storage	International	RU20160111906 20160329
	Processing technology of quick-frozen grape berries	International	CN20151449787 20150728
	Composition of formulation for inner beauty using berries extract as a major ingredient and method thereof	International	KR20150000471 20150105
	Extraction Tool for Stemming Soft Fruit and Berries	International	US201414267044 20140501
	Antioxidant compound jelly with natural berries rich in anthocyanin special for astronauts and preparation method of antioxidant jelly	International	CN20151065873 20150207

(continued)

Table 2 (continued)

Functional food	Patent name	Classification	Application number
Edible berries	Special compressed food prepared by taking berries as raw materials and used for supplementing vitamins to astronauts and processing method of special compressed food	International	CN201410761293 20141213
	Method and device for producing a functional product of berries made into puree with crushed seeds and skins of the berries and the product made by this method	International	EP20150712444 20150218
	Morus berries and avoiding glucose peaks	International	CN20118075610 20111201
	Goji berries extract production method	International	RU20110140748 20111010
	Methods of wild berries fermented powder tablet and wild berries fermented powder tablet manufactured the methods	International	KR20110050626 20110527
	Method for nonwaste low-temperature processing of fruit and berries for preserves	International	RU20090138771 20091020
	Natural antioxidative feed additive based on elements from berries	International	EP20090764174 20091029
	Method of preserving fruits and berries	International	RU20070130948 20070813
Grapes and wine	Improved and more gentle process for extracting useful substances from grapes, grape must extracted therefrom and wine produced therefrom, as well as device for carrying out electroporation	International	ZA20060006882 20060817
	A method of handling harvested grapes and converting same to wine without exposure to oxidizing air	International	ZA19730007718 19731002
Pomegranate	Use of a vegetal formulation containing pomegranate seed oil as an immune system enhancer due to its antioxidant function	International	WO2018TR50157 20180410
	Use of a vegetal formulation containing pomegranate seed oil as an assistive agent in reduction of blood lipids known as triglyceride	International	WO2018TR50158 20180410
	Composition for improving skin condition comprising pomegranate concentrate as active ingredient	International	KR20180153788 20181203
	Pomegranate hand cream and preparation method thereof	International	CN201810646859 20180621
	Skin whitening pomegranate extract and preparation method thereof	International	CN201810603225 20180612

(continued)

Table 2 (continued)

Functional food	Patent name	Classification	Application number
Pomegranate	Pomegranate peel antioxidant additive and preparation method thereof	International	CN20161195941 20161202
	Pomegranate extract for relieving of menopausal symptoms containing a high content of ellagic acid	International	KR20180042898 20180412
	Whitening and moisturizing mask containing pomegranate essence and preparation method of mask	International	CN201711439770 20171227
	Hydrogel sour pomegranate powder skin care product as well as preparation method and application thereof	International	CN201711214270 20171128
	Pomegranate fresh fruit preservation method	International	CN201711030441 20171027
	Preparation method of health-preserving pomegranate tea	International	CN201710848497 20170919
	Method for producing pill for relieving menopausal symptoms containing pomegranate	International	KR20160036782 20160328
	Health care pomegranate tea	International	CN201611017478 20161119
	Red pomegranate anti-allergy anti-inflammatory whitening shower gel and preparation method thereof	International	CN201710104678 20170224
	Preparation method of functional pomegranate ice wine	International	CN201611091452 20161201
	Pomegranate skin extract for treating fatty liver	International	US201415109723 20140109
	Preparation method of pomegranate peel antioxidant substance	International	CN201610367488 20160530
	Application of pomegranate and sour pomegranate medicine composition to preparation of drug for treating hyperlipidaemia	International	CN201610130896 20160308
	Anti-aging natural pomegranate skin-care product and preparation method thereof	International	CN201610189191 20160330
	Pomegranate seed soft capsule	International	CN20151910165 20151210
Nuts	Nuts with added healthy function and method for manufacturing the same	International	KR20180130290 20181029
	Nut-based beverage made with unroasted nuts	International	MX20170014851 20160516
	Nuts with added healthy function and method for manufacturing the same	International	KR20160156225 20161123

(continued)

Table 2 (continued)

Functional food	Patent name	Classification	Application number
Nuts	Process for bleaching nuts Juglans regia L.	International	MD2017S000084 20170704
	Method for extracting total flavonoids in macadamia nuts	International	CN201711267839 20171205
	Dietary therapy formula containing areca nuts and preparation method	International	CN201710356623 20170503
	Antioxidant's survey device in roasted seeds and nuts food	International	CN201720334020U 20170331
	Low-temperature, forced-convection, steam-heating of nuts	International	US201415122824 20140303
	Diet nuts manufacturing method	International	KR20150057515 20150423
	Method for continuous extraction of polyphenol and amygdalin in loquat nuts	International	CN201610446954 20160620
Brassicaceae	Brassicaceae sprout having high content of phytochemical, and method for producing the same	International	JP20160216716 20161104
	Method for increasing sulforaphane contained in vegetable of brassicaceae group	International	JP20150001980 20150108
	Cancer chemoprotective product comprising glucoraphanin and/or glucoraphanen compound and myrosinase enzyme from brassicaceae plant sources	International	AU20110337270 20111129
	Brassicaceae vegetables which angiotensin i converting enzyme inhibitory activity is increased by electrical treatment and products thereof	International	JP20110158381 20110719
	Post-harvest amplification method of active compounds in brassicaceae vegetables using high hydrostatic pressure and brassicaceae vegetables treated with the same method	International	KR20090088387 20090918
	Preparing extract from plant of Brassicaceae, useful e.g. in agrofood composition, comprises crushing and drying plant raw material, subjecting obtained material to extraction by organic solvent and separating liquid fraction of extract	International	FR20090004526 20090923
Spices	A spray -drying device for powdered spices	International	CN201721805575U 20171221
	Be used for health preserving spices to cut up grinder	International	CN201720490099U 20170505
	Acne treatment formula with spices	International	CN201710553510 20170708
	Spices draws enrichment facility	International	CN20152814408U 20151021

(continued)

Table 2 (continued)

Functional food	Patent name	Classification	Application number
Spices	Extraction of essential oils from spices using the microwave technique for the manufacture of biofungicides	International	MX20140007045 20140612
	Essential oil extract of natural spices, encapsulated, oily dispersed and its preparation method	International	PL20110397664 20111230
	Method for improving the aroma of sterilized leaf spices, especially marjoram and thyme	International	PL20090390034 20091223
	Method for improving extraction rate of total flavonoid in spices by using radiation technique	International	CN20101510872 20101018

durations are used, most of them include subjects coming from different populations, sex, age, body mass index, and other features. This approach risks to flattening the observed results that are already sufficiently weakened by the small sample size and by the huge number of bacterial strains/plant extracts or isolated compounds investigated alone or in combination. Basic research suggests a direct correlation between the consumption of polyphenol-rich foods and foods low in fatty acids, such as fruits and vegetables, and the decreased onset of various oxidative stress-related diseases such as cardiovascular and inflammatory diseases, diabetes, and cancer. However, in vitro and in vivo results are not always movable to humans due to the large inter--individual pharmacokinetic variability; hence, it is important to carry out well-designed clinical trials. Therefore, what emerges from the literature currently available is the lack of studies, which investigate in depth the pharmacokinetics of these compounds. In fact, before carrying out a clinical study, a rational formulation of the molecules of interest should be taken into account, which guarantees best bioavailability and consequently health effects.

2.10 Cross-References

- ▶ Caffeoylquinic Acids
- ▶ Coumaric and Cinnamic Acids in Food
- ▶ Dietary Coumarins
- ▶ Organosulfur Compounds in Food
- ▶ Prenylated Flavonoids in Food
- ▶ Tea Catechins
- ▶ Theaflavins, Thearubigins, and Theasinensins

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Dietary Flavonols and O-Glycosides

3

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Contents

Abstract

Flavonols are the most widespread subgroup of the flavonoids. Quercetin, kaempferol, myricetin, and isorhamnetin are the major dietary flavonol aglycones, which most commonly occur as *O*-glycosides in dietary sources including fruits, vegetables, tea, and wine. The role of flavonols and *O*-glycosides

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in human nutrition has gained increased interest due to their associated health beneficial effects for a number of chronic diseases, including cardiovascular diseases, diabetes, and cancer. However, the potential bioactivity of flavonols and *O*-glycosides will depend on their bioavailability. Following digestion, flavonol glycosides are cleaved to their aglycones, which may be metabolized in the enterocytes and further in the liver, forming glucuronidated, sulfated, and/or methylated metabolites, or they may be passively permeate the intestinal epithelial barrier. Flavonols that reach the colon may be degraded by the colonic microbiota to different metabolites, which may also contribute to the observed biological effects. In this chapter, the recent findings on the bioavailability, metabolism, bioactivity, and benefits of dietary flavonols and *O*-glycosides are highlighted. In addition, the recent information on food applications, safety issues, marketed products, and patents are also presented.

Keywords

Quercetin · Kaempferol · Myricetin · Isorhamnetin · Bioavailability · Metabolism · Cardiovascular diseases · Diabetes · Cancer · Toxicity

3.1 Introduction

Flavonols belong to a large group of compounds collectively known as flavonoids, which are a subgroup of an even larger group of compounds known as polyphenols. They are the most widespread subgroup of the flavonoids, being dispersed throughout the plant kingdom with the exceptions of algae and fungi (Crozier et al. 2009). Flavonols not attached to sugar moieties are referred as the aglycone form, whereas flavonols with sugar moieties are called flavonol glycosides. The major dietary flavonol aglycones are quercetin (3,5,7,3′,4′-pentahydroxyflavone), kaempferol (3,5,7,4′-tetrahydroxyflavone), myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone), and isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone), which most commonly occur as *O*-glycosides. Fruits, vegetables, and beverages are important dietary sources of these flavonol *O*-glycosides. In particular, berries, onion, and *Brassica* vegetables including cabbage, kale and broccoli, buckwheat, tea, and red wine are among the well-known sources of quercetin and kaempferol aglycones and *O*-glycosides (Table 1). Apples also contain quercetin glycosides including quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-arabinoside, and quercetin 3-*O*-xyloside. However, as apple flavonols are almost exclusively present in the peel of the fruit (Jakobek and Barron 2016), the flavonol content of whole fruit is low (5.73 mg/100 g fresh weight) compared to berries (www.phenol-explorer.eu) (Table 1).

Dietary intake of flavonols varies between countries. In the Netherlands, the average intake of flavonols was reported to be approximately 23 mg/day, of which quercetin contributed 16 mg/day, kaempferol 3.9 mg/day, and myricetin 1.4 mg/day. Tea was the major source in this population (48% of total intake), followed by onions (29%) and apples (7%) (Hollman and Arts 2000). The same foods and beverages are

Table 1 Dietary sources of flavonols and *O*-glycosides

Category	Food or beverage	Flavonols and *O*-glycosides	Content (mg/100 g FW or mg/100 mL)
Fruits	Black chokeberry	Quercetin 3-*O*-galactoside	46.46
		Quercetin 3-*O*-glucoside	41.95
		Σ	**88.41**
	Black elderberry	Quercetin	**42.00**
	Highbush blueberry	Kaempferol 3-*O*-glucoside	0.62
		Myricetin 3-*O*-arabinoside	12.21
		Myricetin 3-*O*-rhamnoside	1.03
		Quercetin 3-*O*-acetyl-rhamnoside	5.66
		Quercetin 3-*O*-arabinoside	7.09
		Quercetin 3-*O*-galactoside	8.99
		Quercetin 3-*O*-glucoside	1.49
		Quercetin 3-*O*-xyloside	1.60
		Σ	**38.69**
	Lingonberry	Kaempferol	0.53
		Kaempferol 3-*O*-glucoside	1.23
		Quercetin 3-*O*-arabinoside	4.29
		Quercetin 3-*O*-galactoside	13.22
		Quercetin 3-*O*-rhamnoside	12.20
		Σ	**31.47**
	Bog bilberry	Myricetin	13.65
		Quercetin	17.03
		Σ	**30.68**
Vegetables	Red onion	Isorhamnetin	1.51
		Isorhamnetin 4′-*O*-glucoside	6.00
		Quercetin	1.31
		Quercetin 3,4′-*O*-diglucoside	77.08
		Quercetin 3-*O*-glucoside	1.80
		Quercetin 3-*O*-rutinoside	0.21
		Quercetin 4′-*O*-glucoside	38.80
		Quercetin 7,4′-*O*-diglucoside	1.80
		Σ	**128.51**
	Black olive	Quercetin 3-O-rhamnoside	4.07
		Quercetin 3-O-rutinoside	45.36
		Σ	**49.43**
	Chinese cabbage (pak choy)	Kaempferol	9.60
		Myricetin	0.10
		Quercetin	39.00
		Σ	**48.70**
	Kale	Kaempferol	26.74
		Quercetin	7.71
		Σ	**34.45**

(continued)

Table 1 (continued)

Category	Food or beverage	Flavonols and *O*-glycosides	Content (mg/100 g FW or mg/100 mL)
	Chili pepper	Quercetin	**32.59**
	Broccoli	Kaempferol 3,7-*O*-diglucoside	1.50
		Kaempferol 3-*O*-glucoside	1.40
		Kaempferol 3-*O*-sophoroside	16.60
		Quercetin 3-*O*-glucoside	1.80
		Quercetin 3-*O*-sophoroside	6.50
		Σ	**27.80**
Cereals	Buckwheat	Quercetin	0.11
		Quercetin 3-*O*-rutinoside	36.14
		Σ	**36.25**
Pulses	Beans	Kaempferol 3-*O*-acetyl-glucoside	16.40
		Kaempferol 3-*O*-glucoside	39.88
		Kaempferol 3-*O*-xylosyl-glucoside	11.50
		Σ	**67.78**
Seasonings	Caper	Kaempferol	104.29
		Kaempferol 3-*O*-rhamnosyl-rhamnosyl-glucoside	19.53
		Kaempferol 3-*O*-rutinoside	165.76
		Quercetin	32.82
		Quercetin 3-*O*-rutinoside	332.29
		Σ	**654.69**
	Saffron	Kaempferol 3,7,4′-*O*-triglucoside	103.92
		Kaempferol 3-*O*-sophoroside	150.75
		Kaempferol 3-*O*-sophoroside 7-*O*-glucoside	255.32
		Σ	**509.99**
	Mexican oregano	Galangin	188.00
		Methylgalangin	42.07
		Quercetin	42.00
		Σ	**272.07**
Coffee and cocoa	Dark chocolate	Quercetin	**25.00**
Beverages	Black tea	Kaempferol	0.13
		Kaempferol 3-*O*-galactoside	3.08
		Kaempferol 3-*O*-glucoside	12.20
		Kaempferol 3-*O*-glucosyl-rhamnosyl-glucoside	1.03
		Kaempferol 3-*O*-rutinoside	17.36
		Quercetin	0.09
		Quercetin 3-*O*-galactoside	4.17

(continued)

Table 1 (continued)

Category	Food or beverage	Flavonols and *O*-glycosides	Content (mg/100 g FW or mg/100 mL)
		Quercetin 3-*O*-glucoside	10.87
		Quercetin 3-*O*-glucosyl-rhamnosyl-galactoside	0.64
		Quercetin 3-*O*-glucosyl-rhamnosyl-glucoside	6.51
		Quercetin 3-*O*-rhamnoside	0.93
		Quercetin 3-*O*-rutinoside	19.68
		Σ	**76.69**
	Red wine	Isorhamnetin	0.33
		Isorhamnetin 3-*O*-glucoside	0.26
		Kaempferol	0.23
		Kaempferol 3-*O*-glucoside	0.79
		Myricetin	0.83
		Quercetin	0.83
		Quercetin 3-*O*-arabinoside	0.49
		Quercetin 3-*O*-glucoside	1.14
		Quercetin 3-*O*-rhamnoside	1.15
		Quercetin 3-*O*-rutinoside	0.81
		Σ	**6.86**

The data presented in this table is extracted from Phenol-Explorer database (www.phenol-explorer.eu)

also the most predominant dietary sources of flavonols in Denmark and the USA. However, eating habits and cultural differences may significantly influence the dietary source of flavonols. For example, while green tea is the superior source of flavonols in Japan, berries are reported to be the most important dietary sources in Finland. Variations of dietary sources also can be observed among different regions within the same country. For instance, in Italy, red wine is known to be the most significant source of dietary flavonols. On the other hand, in the northern villages of Italy, the major sources of flavonols are reported to be fruits and vegetables (Aherne and O'Brien 2002). In addition to the above, the methods of culinary preparation also have a marked impact on the flavonol content of foods and hence their intake. For example, peeling of fruits and vegetables can eliminate a significant portion of flavonols because these compounds are often present in higher concentrations in the outer parts than in the inner parts, as in case of apples. Moreover, many fresh fruits and vegetables are subjected to a form of processing, e.g., cooking, before consumption, which may also significantly affect the flavonol content. Accordingly, it has been demonstrated that the quercetin content of onions and tomatoes reduced by 75–80% after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying (Briones-Labarca et al. 2011).

In 1936, Szent-Györgyi found that quercetin 3-*O*-rutinoside, also known as rutin, had vitamin properties and termed this flavonol as "vitamin P." At that time, dietary

flavonols are believed to have a poor bioavailability, and therefore nutritional scientists excluded vitamin P from the category of vitamins. In 1970s, quercetin and other plant flavonols are assumed to be potential carcinogens as these compounds were found to possess mutagenic activity (Terao 2009). On the other hand, a great number of recent scientific studies based on in vivo experiments reported the benefits of flavonol consumption with respect to cardiovascular diseases, diabetes, inflammation, viral infections, enhanced physical strength, and cancer prevention (Ahmad et al. 2015; Gormaz et al. 2015; Chen et al. 2016a; Kashyap et al. 2016; Bazzucchi et al. 2019). However, the potential bioactivity of flavonols and *O*-glycosides will depend on their bioavailability. Following digestion, flavonol glycosides are cleaved to their aglycones, which may be metabolized in the enterocytes and further in the liver, forming glucuronidated, sulfated, and/or methylated metabolites, or they may passively permeate the intestinal epithelial barrier (Day et al. 2001). Furthermore, flavonols that reach the colon may be degraded by the colonic microbiota to different phenolic acids, e.g., phenylacetic acid and protocatechuic acid (Serra et al. 2012), which may also contribute to the observed biological effects.

Considering the above, in this chapter, bioactive constituents, bioavailability, metabolism, bioactivity, and benefits of dietary flavonols and *O*-glycosides are highlighted. In addition, the recent information on food applications, safety issues, marketed products, and patents are also presented.

3.2 Bioactive Constituents

Flavonols are formed from the combination of derivatives synthesized from phenylalanine via the shikimic acid pathway and acetic acid. The initial step of flavonol biosynthesis includes the formation of amino acid phenylalanine from phenylpyruvate. Then, by the action of phenylalanine ammonia-lyase (PAL) enzyme, phenylalanine is transformed to *trans*-cinnamic acid, which is hydrolyzed to *p*-coumaric acid (C9). The C9 acids condense with three malonyl-CoA molecules (C2) by chalcone synthase (CHS) to form chalcones (C15) (Aherne and O'Brien 2002; Survay et al. 2011). The C15 chalcones are then isomerized into (2*S*)-flavanones by chalcone isomerase (CHI). (2*R*, 3*R*)-*trans*-dihydroflavonols are subsequently formed from (2*S*)-flavanones by flavanone 3β-hydroxylase (FHT). Lastly, flavonol synthase (FLS), 2-oxoglutarate-dependent dioxygenase, catalyzes the desaturation of dihydroflavonols to flavonols (Leonard et al. 2006).

The structure of the flavonols is based on the flavonoid nucleus, which consists of three phenolic rings referred to as the A, B, and C rings. The benzene ring A is condensed with a six-member ring C, which carries a phenyl benzene ring B as a substituent in the 2-position. The term *4-oxo-flavonoids* is often used to describe the structure of flavonols (Aherne and O'Brien 2002), which can be distinguished from other flavonoids with the presence of (i) a double bond at the 2–3-position, (ii) a carbonyl group at the 4-position, and (iii) a hydroxyl group at the 3-position of C ring (Fig. 1). Azaleatin, fisetin, galangin, gossypetin, kaempferide, kaempferol,

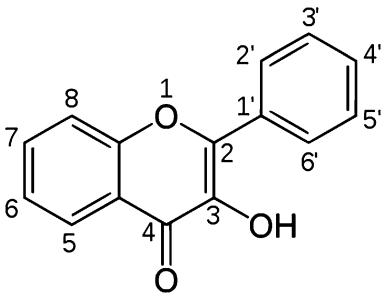

Fig. 1 Chemical structure of flavonols

isorhamnetin, morin, myricetin, natsudaidain, pachypodol, quercetin, rhamnazin, and rhamnetin are the common flavonol aglycones, which structurally differ from each other by variations in the number and position of hydroxyl and methyl groups (Table 2).

In nature, the majority of flavonols are present as *O*-glycosides. Glycosylation occurs frequently at the 3-position of the C ring, but substitutions can also take place at the 5-, 7-, 4′-, 3′-, and 5′-carbons (Crozier et al. 2009). The glycosidic sugars are generally glucose; however other carbohydrate substitutions including arabinose, galactose, rutinose, lignin, rhamnose, and xylose are also present (Aherne and O'Brien 2002). There are several flavonol glycosides, comprising mono-, di-, and tri-glycosides based upon quercetin, kaempferol, azaleatin, kaempferide, myricetin, and rhamnetin, and various permutations of glucose, galactose, rhamnose, arabinose, and rutinose. Some examples of flavonol glycosides and their glycosylation positions are given in Table 3; however it is noteworthy to mention that there are numerous flavonol conjugates, with 179 different glycosides of quercetin alone (Hollman and Arts 2000). The classification of the structures requires the knowledge of the nature of α or β aglycon sugar bonds as well as the optical configuration of the involved sugar (dextro or levo). As a rule, D-configured sugars, i.e., glucose, galactose, xylose, and glucuronic acid, form β bonds, while the α bonds are made of L-sugars such as arabinose and rhamnose. For example, this arrangement can be observed in the composition of apple flavonols, which are composed of quercetin glycosides, including α-L-arabinoside, β-D-galactoside, β-D-glucoside, α-L-rhamnoside, and β-D-xyloside (Escarpa and Gonzalez 2001).

3.3 Bioavailability and Metabolism

Several factors affect the bioavailability of a compound, including degradation by gut microflora and enzymes, decomposition in the gut lumen, and first pass metabolism by the liver. In addition, the physicochemical properties of the compound will also influence its bioavailability, consisting molecular weight, partition coefficient, and pKa (Choudhury et al. 1999). The absorption of dietary aglycones and *O*-glycosides has been the subject of many studies, and most of them have been related with quercetin and its glycosides.

Table 2 Flavonols and their hydroxylation and methylation positions

Flavonol	IUPAC name	5	6	7	8	2′	3′	4′	5′	6′
Azaleatin	2-(3,4-dihydroxyphenyl)-3,7-Dihydroxy-5-methoxychromen-4-one	OCH_3	H	OH	H	H	H	OH	OH	H
Fisetin	3,3′,4′,7-Tetrahydroxy-2-phenylchromen-4-one	H	H	OH	H	H	OH	OH	H	H
Galangin	3,5,7-Trihydroxy-2-phenylchromen-4-one	OH	H	OH	H	H	H	H	H	H
Gossypetin	2-(3,4-dihydroxyphenyl)-3,5,7,8-Tetrahydroxychromen-4-one	OH	H	OH	OH	H	OH	OH	H	H
Kaempferide	3,5,7-Trihydroxy-2-(4-methoxyphenyl) chromen-4-one	OH	H	OH	H	H	H	OCH_3	H	H
Kaempferol	3,4′,5,7-Tetrahydroxy-2-phenylchromen-4-one	OH	H	OH	H	H	H	OH	H	H
Isorhamnetin	3,5,7-Trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one	OH	H	OH	H	H	OCH_3	OH	H	H
Morin	2-(2,4-dihydroxyphenyl)-3,5,7-Trihydroxychromen-4-one	OH	H	OH	H	OH	H	OH	H	H
Myricetin	3,3′,4′,5′,5,7-Hexahydroxy-2-phenylchromen-4-one	OH	H	OH	H	H	OH	OH	OH	H
Natsudaidain	2-(3,4-dimethoxyphenyl)-3-Hydroxy-5,6,7,8-tetramethoxychromen-4-one	OCH_3	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H
Pachypodol	5-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,7-dimethoxychromen-4-one	OH	H	OCH_3	H	H	OCH_3	OH	H	H
Quercetin	3,3′,4′,5,7-Pentahydroxy-2-phenylchromen-4-one	OH	H	OH	H	H	OH	OH	H	H
Rhamnazin	3,5-Dihydroxy-2-(4-hydroxy-3-	OH	H	OCH_3	H	H	OCH_3	OH	H	H

(continued)

Table 2 (continued)

Flavonol	IUPAC name	5	6	7	8	2′	3′	4′	5′	6′
	methoxyphenyl)-7-methoxychromen-4-one									
Rhamnetin	2-(3,4-dihydroxyphenyl)-3,5-Dihydroxy-7-methoxychromen-4-one	OH	H	OCH_3	H	H	OH	OH	H	H

Reproduced from Survay et al. (2011)

Table 3 Flavonol glycosides and their glycosylation positions

Aglycone	Flavonol glycoside	3	7	8	3′	4′
Azaleatin	Azalein	Rhamnose				
Kaempferide	Icariin	Rhamnose	Glucose	tert-Amyl		
Kaempferol	Amurensin		Glucose	tert-Amyl		
	Astragalin	Glucose				
	Kaempferitrin	Rhamnose	Rhamnose			
	Robinin	Robinose	Rhamnose			
Myricetin	Myricitrin	Rhamnose				
Quercetin	Hyperoside	Galactose				
	Isoquercetin	Glucose				
	Quercitrin	Rhamnose				
	Rutin	Rutinose				
	Spiraeoside					Glucose
	Troxerutin	Rutinose	Hydroxyethyl		Hydroxyethyl	Hydroxyethyl
Rhamnetin	Xanthorhamnin	Trisaccharide				

Reproduced from Survay et al. (2011)

The bound sugar moiety is also known to influence the bioavailability of flavonoids. For instance, Morand et al. (2000) examined the effect of the type/nature of the sugar on the absorption of glycosides. They found that quercetin 3-glucose (33.2 μM) absorbed in the small intestine and was better absorbed than quercetin (11.7 μM) itself. In contrast, glycosides containing a rhamnose moiety could not be absorbed in the small intestine. These researchers suggested that the 3-O-glucosylation improves the absorption of quercetin in the small intestine.

In another study, Manach et al. (1995) studied the bioavailability and the plasma transport of flavonols in rats fed with quercetin or rutin diets. They also investigated the flavonol concentrations in plasma, ileal and cecal contents, and feces. They suggested that the rate of elimination of quercetin metabolites was relatively low and

high plasma concentrations can be easily maintained with a regular supply of flavonoids in the diet. In contrast, Gugler et al. (1975) showed that quercetin was poorly absorbed (1%) across the gastrointestinal tract in a human trial. However, they observed that recovery in feces after the oral dose was 53% degraded by microorganisms in the gut.

Choudhury et al. (1999) investigated the absorption and excretion of the aglycone quercetin and compared with its 3-glucoside (isoquercitrin) and 3-rhamnoglucoside (rutin) in the rat. They reported that isoquercitrin (0.48% of administered dose) orally absorbed was bioavailable. On the other hand, their results revealed that neither unchanged rutin, quercetin, nor the conjugated metabolites in the form of glucuronide or sulfate were detected in the urine after oral dosing. They also found that all the flavonoids studied produced low total urinary recoveries after intravenous administration, 9.2% for quercetin-3-rhamnoglucoside, 6.7% for the 3-glucoside, and 2.4% for the aglycone, indicating that extensive metabolism to low molecular weight compounds or excretion via other routes may be occurring. The differences in the metabolism of each compound could be linked to their physicochemical properties. Quercetin is the most lipophilic compound enabling its entry by passive diffusion into the liver. Very little unchanged quercetin and a greater number of metabolites and their conjugates were observed in the urine. However, rutin (less lipophilic compound) would not be distributed into the liver as readily. This lead to a greater proportion of unchanged compound and fewer metabolites being detected in the urine (Choudhury et al. 1999). Erlund et al. (2001) showed that quercetin-3-rutinoside was more bioavailable in women compared to men, and plasma levels were the highest in women using oral contraceptives. This could be related to interindividual variation in the gastrointestinal microflora or absorption or biotransformation mechanisms which affect the bioavailability. Another study conducted by Hollman et al. (1999) reported that the peak concentration of quercetin (C_{max}) in plasma was 20 times higher and reached T_{max} more than 10 times faster after the intake of glucoside ($C_{max} = 3.5$ μM; $T_{max} < 0.5$ h) than the rutinoside ($C_{max} = 0.18$ μM; $T_{max} = 6.0$ h). They also suggested that quercetin glucoside is actively absorbed from the small intestine, whereas quercetin rutinoside is absorbed from the colon after deglycosylation. Reinboth et al. (2010) investigated the bioavailability of quercetin in dogs, administering oral doses of 30 mmol/kg b.w. of the aglycone, isoquercitrin, or rutin, equivalent to 10 mg quercetin/kg b.w. They have reported that quercetin and isoquercitrin were mainly absorbed in the small intestine with isoquercitrin being one and a half times more bioavailable than quercetin. Dang et al. (2014) indicated that the bioavailability of myricetin was found to be 9.62% and 9.74% at two oral doses (50 mg/kg and 100 mg/kg, respectively), showing that it was poorly absorbed after oral administration.

In general, the first part of metabolism consists of tissues including the small intestine, liver, and kidneys, whereas the second part of the metabolism occurs in the colon. Studies on humans have clearly shown that metabolism in human body is vital to determine which ones are better absorbed and which ones cause formation of bioactive metabolites. Sugar moieties, such as quercetin-3-glucoside, are cleaved from the phenolic backbone in the small intestine and absorbed here through the

ingestion of flavonoids. Moreover, enzymes play an important role in the absorption. For instance, lactase phlorizin hydrolase (at enterocyte membrane) or β-glucosidase (cytosolic, for polar glycosides) hydrolyze glycosylated flavonoids and then aglycones enter epithelial cells by passive diffusion. On the other hand, some flavonoids linked to a rhamnose moiety must reach the colon and hydrolyzed by the colon microbiota (Marín et al. 2015). When the aglycon or the new bioactive metabolites formed are absorbed at small intestine, they go through some degree of phase II metabolism at enterocyte level, such as glucuronidation, methylation, and sulfation (Hollman 2004; Marín et al. 2015). Afterward, these products enter the bloodstream by the portal vein, reaching the liver. If a flavonoid glycoside is not absorbed in the small intestine, it can be metabolized by the colonic microflora into its aglycone in the large intestine (Xiao 2017). It is also worth emphasizing that tissue distribution can help predict a variety of events related to the efficacy. Xu et al. (2014), for example, reported that the highest level of kaempferide was observed in heart, whereas tamarixetin was observed in the lung of rats when their distribution in different tissues (heart, liver, lung, spleen, kidney, prostate, and brain) was investigated.

3.4 Bioactivities

Dietary flavonols and *O*-glycosides have many important biological activities, including antioxidant, anti-inflammatory, antiangiogenic, hypolipidemic, neuroprotective, and anticancer effects (Table 4). All these studies significantly indicated that dietary flavonols and *O*-glycosides may help treating some critical health problems.

Epilepsy is a common neurological disorder in which brain activity becomes abnormal, causing unpredictable seizures. Das et al. (2017), after investigating the effects of fisetin in traumatic epilepsy in rats for its antiepileptic activity, reported that fisetin pretreatment was found to inhibit the development of iron-induced electrical seizure and significantly decrease the corresponding multiple unit activity in the cortex and in the hippocampus. Moreover, it significantly reduced the production of malondialdehyde suggesting the antilipidperoxidative action of fisetin. Another worldwide health problem is food allergy for which present medications have many side effects and do not stop the progression of hypersensitivity reactions. Elkholy et al. (2019) showed that fisetin exerted potent immunomodulatory and anti-inflammatory activities, thus abled them to alleviate OVA-induced food allergy in mice. This alleviation could be due to their capability to rebuild the normal T-helper 1/T-helper 2 cytokine balance and AT1 blockade activity. In addition, Kim et al. (2014b) reported that administration of spiraeoside to mice suppressed the passive cutaneous anaphylaxis reaction. Thus, they supposed that spiraeoside can be utilized as an anti-allergic agent.

Even though there are many researches on acute lung injury and acute respiratory distress syndrome, the mortality rate as a result of these diseases still remains high. In the study of Feng et al. (2016), fisetin was injected (1, 2, and 4 mg/kg, i.v.) 30 min

Table 4 Recent animal studies on chronic disease

Components	Chronic disease	Animal model	Key outcomes	References
Fisetin	Epilepsy	Wistar rats	Antiepileptic action of fisetin in iron-induced model of epileptic rats by inhibiting oxidative stress	Das et al. (2017)
	Food allergy	BALB/c mice	Fisetin alleviated food allergy and shows potent anti-inflammatory and immunomodulatory effects	Elkholy et al. (2019)
	Acute lung injury	Sprague-Dawley rats	Fisetin may regulate the inflammatory process in lipopolysaccharide-induced acute lung injury	Feng et al. (2016)
	Ischemic heart disease	Wistar rats	Fisetin attenuates isoproterenol-induced cardiac ischemic injury	Garg et al. 2019
	Neurotoxicity	Pregnant Wistar rats	Fisetin reduced the toxic effects of MeHg in the developing rat brain	Jacob and Thangarajan (2017)
	Depression	ICR mice Ahi1$^{loxp/loxp}$ mice	Fisetin provided antidepressant effects	Wang et al. (2017)
	Acute pancreatitis	C57BL/6mice	Fisetin reduced pancreatitis and pancreatitis-associated lung injury	Jo et al. (2014)
	Atopic dermatitis	NC/Nga mice	Fisetin exhibited as a potential therapeutic for atopic dermatitis	Kim et al. (2014)
	Alcohol-induced acute liver	C57BL/6 mice	Fisetin at 5 and 10 mg/kg can attenuate the adverse effects of alcohol in liver tissues	Koneru et al. (2016)
	Glaucoma	DBA/2 J mouse	Fisetin was able to promote the visual functions of DBA/2 J mice	Li et al. (2019)
	Cardiac hypertrophy	SHR and control Wistar Kyoto rats	Fisetin inhibited cardiac hypertrophy in hypertension rats	Lin et al. (2019)
	Hyperglycemia	Wistar rats	Fisetin ameliorated hyperglycemia in diabetic rats	Althunibat et al. (2019)
	Aging	Wistar rats	Fisetin could be considered as an antiaging compound	Singh et al. 2019
Galangin	Hypertrophic scar	BALB/c mice	Galangin was a novel anti-hypertrophic scar compound	Zhang et al. (2016)

(continued)

Table 4 (continued)

Components	Chronic disease	Animal model	Key outcomes	References
Hyperoside	Osteoporosis	Kunming mice	Hyperoside was effective in preventing osteoporosis	Chen et al. (2018)
	Chronic liver fibrosis	Kunming mice	Hyperoside has a potential anti-fibrosis and protective physiological function of livers	Zou et al. (2017)
Isoquercetin	Diabetes mellitus	Wistar rats	Isoquercetin regulated nuclear factor erythroid 2-related factor 2, inflammatory, and AMP-activated protein kinase pathway genes	Jayachandran et al. (2019)
	Cerebral ischemic stroke	Sprague-Dawley rats	Isoquercetin has a neuroprotective effect against I/R brain injury	Dai et al. (2018)
Isoquercitrin	Liver injury	Kunming mice	Isoquercitrin protected liver from APAP induced injury	Xie et al. (2016)
	Type 2 diabetes mellitus	Kunming mice	Isoquercitrin significantly inhibited postprandial blood glucose changes in a dose-dependent manner	Zhang et al. (2018)
Kaempferol	Myocardial ischemia/ reperfusion injury	Sprague-Dawley rats	Kaempferol provided cardioprotection via antioxidant activity and inhibition of phospho-GSK-3β activity in rats with I/R	Zhou et al. (2015)
Morin	Schizophrenia	Swiss mice	Morin exhibited antipsychotic-like activity	Ben-Azu et al. (2018)
Myricetin	Ischemic stroke	Sprague-Dawley rats	Myricetin attenuated brain injury and neurological deficits	Wu et al. (2016)
Myricitrin	Liver damage	BALB/ cN mice	Myricitrin showed a significant hepatoprotective activity	Domitrović et al. (2015)
Quercetin	Rheumatoid arthritis	C57BL/6 mice	Quercetin decreased the severity of arthritic inflammation, protecting joints from degradation	Haleagrahara et al. (2017)
	Liver injury	ICR mice	Quercetin inhibited CCl_4-induced liver injury	Ma et al. (2015a)
	Liver injury	Kunming mice	Quercetin showed a potential protective effect against perfluorooctanoic acid-induced liver damage via attenuation of oxidative stress and inflammation	Zou et al. (2015)

(continued)

Table 4 (continued)

Components	Chronic disease	Animal model	Key outcomes	References
Spiraeoside	Type I allergy	Balb/c mice	Spiraeoside prevented mast cell activation and allergic responses	Kim et al. (2014)
Troxerutin	Hepatic gluconeogenesis	ICR mice	Troxerutin markedly reduced high-fat diet-induced enhancement of hepatic gluconeogenesis	Zhang et al. (2017)
	Liver cancer	Wistar rats	Troxerutin exerted a significant therapeutic effect against liver cancer by modulating liver function enzymes, xenobiotic enzymes	Thomas et al. (2017)
Icariin	Parkinson's disease	C57BL/6 mice	Icariin showed neuroprotective effect on dopaminergic neurons in Parkinson's disease mice	Chen et al. (2017)
	Bone infection	Rabbits	Icariin exhibited osteoplastic properties on osteoblasts and enhanced bone repair	Zhang et al. (2017)
	Depression	Sprague-Dawley rats	Icariin showed protective effects against corticosterone-induced depression and metabolic dysfunction	Gong et al. (2016)
	Ischemic stroke	Sprague-Dawley rats	Icariin exhibited neuroprotective effect on ischemic stroke in rats	Xiong et al. (2016)
	Acute kidney injury	C57BL/6 N mice	Icariin protected against kidney injury	Xie et al. (2018)

before lipopolysaccharide (LPS) administration (5 mg/kg, i.v.) in rats. They demonstrated that fisetin effectively reduced the inflammatory cytokine release and total protein in bronchoalveolar lavage fluids and the lung wet/dry ratios. Fisetin, moreover, inhibited LPS-induced increases of neutrophils and macrophage infiltration and attenuated myeloperoxidase activity in lung tissues. Additionally, the protective effect of fisetin in acute lung injury may be due to its ability to inhibit the expression Toll-like receptor 4 and the activation of nuclear factor-κB (NF-κB) in lung tissues. In addition, Jo et al. (2014) showed that pre- and posttreatment with fisetin attenuated the severity of cerulein-induced pancreatitis and pancreatitis-associated lung injury, inhibiting the activation of NF-κB and c-Jun NH_2-terminal kinase. Atopic dermatitis (AD) is a chronically relapsing and pruritic inflammatory skin disease. Kim et al. (2014a) investigated whether fisetin relieves AD-like clinical symptoms induced by repeated dinitrofluorobenzene treatment in NC/Nga mice. They reported

that fisetin significantly inhibited infiltration of inflammatory cells and suppressed the expressions of various inflammatory mediators. Furthermore, fisetin also inhibited phosphorylation of NF-κB p65, which associated with inflammation. Recent researches on alcohol consumption indicated that production of reactive oxygen species (ROS) and improvement of lipid peroxidation are the prime causes in the initiation of acute liver injury. Furthermore, the involvement of matrix metalloproteinases (MMPs) plays role in the pathogenesis of alcoholic liver diseases. Koneru et al. (2016), for instance, investigated the protective effect of fisetin on the liver from binge alcohol-induced toxicity and to explore the underlying mechanisms of inhibition of MMP and oxidative stress. They demonstrated that pretreatment with fisetin (5 and 10 mg/kg) ameliorated the alcohol-induced alterations in liver function, antioxidant defense, histological changes, mitochondrial respiratory enzymes, and MMP activities.

Coronary microvascular disease is another type of ischemic heart disease in its severest form, i.e., myocardial infarction, which continues to be a major cause of morbidity and mortality all throughout the world. Garg et al. (2019) reported via an in vivo study that flavonols like fisetin had a cardioprotective effects in the heart at doses of 10 and 20 mg/kg via suppression of oxidative stress-mediated apoptosis and inflammation and suppressed the receptor for advanced glycation end products (RAGE)/NF-κB. Methyl mercury (MeHg) is a neurotoxin causing irreversible cognitive damage in offspring of gestationally exposed mothers. Accordingly, Jacob and Thangarajan (2017) evaluated the effect of gestational intake of fisetin on MeHg neurotoxicity in F1 generation rats. They reported that intake of fisetin during pregnancy in rats ameliorated in utero MeHg exposure induced neurotoxicity outcomes in postnatal weaning F1 generation rats. Same group further investigated the mechanism behind the mitigating action of fisetin against prenatal MeHg exposure-induced neurotoxicity. They concluded that fisetin regulates the expression of regulatory genes and proteins involved in plasticity and synaptic transmission and decreases MeHg neurotoxicity in the developing rat brain (Jacob and Sumathi 2019). Fisetin has been reported to play an important role in depression, causing a series of physiological abnormalities including decreased tropomyosin receptor kinase B (TrkB) phosphorylation. Wang et al. (2017) indicated that fisetin increased phosphorylated TrkB level without altering total TrkB. Gong et al. (2016) also carried out studies on antidepressant-like effect and the possible mechanisms of icariin in a rat model of corticosterone-induced depression. They observed that icariin remarkably increased sucrose intake and hippocampal brain-derived neurotrophic factor levels and decreased the immobility time in forced swim test in corticosterone-induced depressive rats. Icariin pretreatments reversed the pathological process of corticosterone-induced depression via regulation of the disturbed metabolic pathways.

Glaucoma is another common chronic neurodegenerative disease, which could cause visual loss, especially in aged people. Li et al. (2019) carried out studies on whether fisetin is able to mitigate glaucoma. They reported that fisetin is able to promote the visual functions of DBA/2 J mice by inhibiting NF-κB activation. In addition, they found that both mRNA levels and secretory of tumor necrosis

factor alpha (TNFα), interleukin (IL-1β), and IL-6 were dramatically decreased in fisetin-treated DBA/2 J mice compared to untreated mice. In a recent study, Lin et al. (2019) investigated the effect of fisetin extenuates hypertension-associated cardiac hypertrophy in spontaneously hypertension rats. They showed that fisetin inhibits cardiac hypertrophy by effectively suppressing calcineurin-NFATC3, hypertrophic marker, in SHR hearts. In a recent study, Althunibat et al. (2019) observed that fisetin prevented cardiomyopathy via restoration of hyperglycemia and attenuation of oxidative stress, inflammation, and apoptosis in the diabetic heart. In another recent study, Shi et al. (2018b) found that the underlying mechanisms of fisetin could be attributable to suppression of NF-κB and activation of the nuclear factor erythroid 2-related factor 2 pathway. In parallel with these studies, Jayachandran et al. (2019) investigated the effects of streptozotocin (STZ) on Nrf2, NF-kB, and AMPK pathway and how the isoquercetin treatment at a molecular level overcame the burden of diabetes mellitus. They showed that isoquercetin prevented the oxidative stress and regulation of the expression of Nrf2 pathway-associated proteins and genes.

Aging is the time-related deterioration, characterized by different molecular hallmarks at the cellular and organismal level. More recently, Singh et al. (2019) observed that fisetin suppressed the aging-induced elevation in levels of reactive oxygen species, eryptosis, lipid peroxidation, and protein oxidation, whereas it significantly increases the levels of antioxidants and activates plasma membrane redox system. Based on these findings, the authors proposed that fisetin-rich diet might be a potential antiaging intervention strategy.

Hypertrophic scar is a complex fibroproliferative disorder, causing pain, burning, and itching. Zhang et al. (2016) revealed that galangin effectively extenuated hypertrophic scar formation, suppressing proliferation and inhibiting activin receptor-like kinase 5/Smad2/3 signaling, thus suggesting that galangin is as a potential agent for the treatment of hypertrophic scar or other fibroproliferative disorders.

Osteoporosis is a bone disease, causing loss in bone mass, micro-architectural deterioration of bone tissue, and predisposition to fracture. Accordingly, Chen et al. (2018) concluded that hyperoside was effective in preventing osteoporosis, inhibiting the TNF-receptor-associated factor 6, mediating receptor activator of nuclear factor-κB ligand (RANKL)/RANK/NF-κB signaling pathway and elevating the osteoprotegerin/RANKL ratio. Another study conducted by Zou et al. (2017) demonstrated that hyperoside has hepatoprotective action upon CCl_4-induced chronic liver fibrosis in mice, improving the expression of Nrf2 in nucleus, decreasing the MDA content and GOT/GPT/serum monoamine oxidase activity, and increasing the anti-oxidase (GSH-Px/SOD/CAT) activity toward the formation of trichloromethyl radicals.

Ischemic stroke, leading cause of death, is characterized by an obstruction within a blood vessel supplying blood to the brain. Dai et al. (2018) investigated the effect of isoquercetin on ischemia/reperfusion (I/R) brain injury. They found that isoquercetin has a neuroprotective effect against I/R injury in vivo, mediated by extenuating both oxidative stress and neuronal apoptosis via Nrf2-mediated

inhibition of the NOX4/ROS/NF-κB pathway. Similarly, Zhou et al. (2015) found that kaempferol provided cardioprotection via antioxidant activity and inhibition of phospho-GSK-3β activity in rats with I/R. Moreover, Wu et al. (2016) observed that administration of myricetin mitigated brain injury and neurological deficits via improvement of mitochondrial function and activation of Nrf2 pathway. In addition, Xiong et al. (2016) showed that icariin has neuroprotective effect on ischemic stroke in rats, inhibiting inflammatory responses mediated by NF-κB and peroxisome proliferator-activated receptors (PPARα and PPARγ).

Acetaminophen (APAP) causes serious liver damage. Recently, many researches have been performed in mice for their ability to prevent APAP-induced liver injury. Xie et al. (2016) reported that isoquercitrin pretreatments effectively extenuated APAP-induced hepatic oxidative stress via inhibition NF-κB and MAPK pathways and amelioration of iNOS, TNF-α, IL-1β, and IL-6 production. Accordingly, Domitrović et al. (2015) reached similar results; they reported that myricitrin ameliorated toxic liver damage by several mechanisms, increasing glutathione, cytochrome P450 2E1 level, proliferating cell nuclear antigen expression in regenerating liver tissue and reducing hepatic lipid peroxidation, cyclooxygenase-2, and tumor necrosis factor-alpha overexpression and inflammation in the liver, and inhibiting hepatic expression of transforming growth factor-beta1, alpha-smooth muscle actin, and liver fibrosis. Another study conducted by Ma et al. (2015a) demonstrated that quercetin prevented the CCl_4-induced inflammation via modulation of the TLR2/TLR4 and MAPK/NF-κB signaling pathway.

Schizophrenia is a chronic and severe mental disorder. Ben-Azu et al. (2018) examined the possible mechanisms involved in the antipsychotic-like activity of morin in ketamine model of schizophrenia and observed that morin may demonstrate antipsychotic-like therapeutic effect via modulation of oxidative/nitrergic, cholinergic actions and neuroprotection.

Rheumatoid arthritis is a chronic inflammatory disease. Haleagrahara et al. (2017) showed that quercetin reduced the severity of arthritic inflammation disease in animals, decreasing levels of TNF-α, IL-1β, IL-17, and MCP-1.

Zhang et al. (2017) showed that troxerutin effectively decreased high-fat diet-induced enhancement of hepatic gluconeogenesis via its inhibitory effects on endoplasmic reticulum stress-mediated nucleotide oligomerization domain activation and consequent inflammation.

Parkinson's disease is a neurodegenerative disorder, which is related to the dysfunctions of nigrostriatal dopaminergic systems. Chen et al. (2017) examined the neuroprotective effects of icariin on dopaminergic neurons and the possible mechanisms of Parkinson's disease. They reported that icariin has neuroprotective effect on dopaminergic neurons in Parkinson's disease mice model. The potential mechanisms might be related to PI3K/Akt and MEK/ERK pathways.

Acute kidney injury is a complication of sepsis and increases mortality. Xie et al. (2018) research group indicated that icariin ameliorated cecal ligation and perforation-induced mortality and acute kidney injury, inhibiting renal oxidant damage, inflammatory responses, apoptosis, and vascular permeability.

3.5 Benefits

Studies on humans have clearly shown that drug treatment for diseases often causes severe side effects. Therefore, natural compounds, dietary flavonols and *O*-glycosides, exert a significant therapeutic effect against inflammatory, cancer, and diabetes. However, the protective effects of flavonols and *O*-glycosides on human studies are limited, whereas human cell studies are more common (Table 5).

Osteoarthritis is a common disorder, causing pain and stiffness. Moreover, inflammatory cytokines, IL-1β, induce the production of other inflammatory mediators such as NO and PGE2. Ma et al. (2015b) after investigating the anti-inflammatory effects and mechanisms of astragalin on IL-1β-stimulated human osteoarthritis chondrocytes reported that astragalin inhibited IL-1β-induced NO and PGE2 production by activating PPAR-γ, which subsequently inhibited IL-1β-induced NF-κB and MAPK activation in chondrocytes.

Colorectal cancer is the second leading cause of cancer death in women and the third for men. Chemotherapy, which is the most widely used treatment for cancer, may induce a wide range of adverse effects in patients. Recently, Farsad-Naeimi et al. (2018) investigated the effects of fisetin supplementation on inflammatory and metastatic factors in colorectal cancer patients. They demonstrated that fisetin supplementation markedly attenuated the plasma levels of interleukin (IL)-8 and high-sensitivity C-reactive protein. Therefore, fisetin supplementation can ameliorate the inflammatory status in colorectal cancer patients. In another study, Youns and Hegazy (2017) showed that fisetin inhibited cellular proliferation and viability of hepatic (HepG-2, IC50: 3.2 μM), colorectal (Caco-2, IC50: 16.4 μM), and pancreatic (Suit-2, IC50:8.1 μM) cancer cell lines mediated through activation of CDKN1A, SEMA3E, GADD45B, and GADD45A and downregulation of TOP2A, KIF20A, CCNB2, and CCNB1 genes. Moreover, Wang and Huang (2018) suggested that fisetin effectively inhibited cell proliferation and migration and induced apoptosis in NSCLCs. Huang et al. (2015) showed that galangin and myricetin inhibit angiogenesis induced by ovarian cancer cell lines, inhibiting secretion of vascular endothelial growth factor by the Akt/p70S6K/ hypoxia-inducible factor-1α (HIF-1α) pathway. Another study conducted by Zou and Xu (2018) investigated the effects of galangin on the suppression of retinoblastoma. They observed that galangin suppressed the growth of retinoblastoma tumor through inactivating protein kinase B and promoting Caspase-3 pathway. Thyroid cancer is a thyroid malignant tumor causing enlarged lymph node and pain in the anterior region of the neck. More recently, Fang et al. (2019) demonstrated that icariin showed antitumor effect via downregulating miR-625-3p. Moreover, icariin blocked phosphoinositide 3-kinase/protein kinase B and mitogen-activated protein extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase signaling pathways. Another study found that icariin inhibits both inducible and constitutive signal transducer and activator of transcription 3 activation, making suppressor of tumor cell survival, angiogenesis, and proliferation (Jung et al. 2018). Cisplatin is a valuable chemotherapy agent in clinical studies. However, adverse side effects notably nephrotoxicity limit the use of cisplatin which seriously limits

Table 5 Recent human/cell studies on chronic disease

Components	Chronic disease	Human/cell model	Key outcomes	References
Astragalin	Osteoarthritis	25 patients (age, 57)	Astragalin inhibited IL-1β-induced inflammatory mediators NO and PGE2 production in chondrocytes	Ma et al. (2015b)
Fisetin	Colorectal cancer	37 colorectal cancer patients (100 mg fisetin ($n = 18$) or placebo ($n = 19$) for 7 consecutive weeks)	Fisetin could improve the inflammatory status in colorectal cancer patients	Farsad-Naeimi et al. (2018)
	Hepatic, colorectal, and pancreatic cancer	Hepatic (HepG-2), colorectal (Caco-2) and pancreatic (Suit-2) cancer cell lines	Fisetin inhibited cellular proliferation and viability of hepatic, colorectal, and pancreatic cancer cell lines	Youns and Hegazy (2017)
	Lung cancer	A549 human NSCLC cell line	Fisetin inhibited the growth of A549 cells in a dose- and time-dependent manner	Wang and Huang (2018)
	Chronic obstructive pulmonary disease	NCI-H292 (CRL-1848) and HEK293T (CRL-3216)	Fisetin is a good therapeutic drug candidate for the treatment of inflammatory lung diseases	Lee et al. (2018)
Galangin	Ovarian cancer	A2780/CP70 and OVCAR-3	Galangin inhibited angiogenesis induced by ovarian cancer cell lines	Huang et al. (2015)
	Osteosarcoma	MG-63 and U2-OS	Galangin attenuated osteosarcoma cell proliferation	Liu et al. (2017)
	Laryngeal cancer	TU212 and M4e	Galangin suppressed laryngeal cancer cell proliferation, migration, and invasion, inactivating PI3K/AKT and p38 signaling pathways	Wang and Tang (2017)
	Kidney cancer	A498 cells	Galangin inhibited the proliferation of the kidney cancer A498 cells via inhibition of the PI3K/AKT/mTOR signaling pathway	Zhu et al. (2018)

(continued)

Table 5 (continued)

Components	Chronic disease	Human/cell model	Key outcomes	References
	Retinoblastoma	Y-79, C-33A, WERI-Rb-1, ARPE-19	Galangin exhibited a suppressive effect on human retinoblastoma cell proliferation and migration	Zou and Xu (2018)
Icariin	Bipolar disorder Alcohol use disorder	Ten participants with bipolar I or bipolar II disorders	Icariin may reduce depressive symptoms and decrease alcohol consumption	Xiao et al. (2016)
	Cisplatin-induced cytotoxicity	HEK-293 cell	Icariin exerted protective effect on cisplatin-induced cytotoxicity	Zhou et al. (2019)
	Thyroid cancer	SW579 and TPC1 cells	Icariin showed antitumor effect on thyroid cancer	Fang et al. (2019)
	Myeloma	MMcell and U266	Icariin exhibited as a signal transducer and activator of transcription 3 blocker in myeloma therapy	Jung et al. (2018)
Isoquercitrin	Bladder cancer	5637 and T24 cells	Isoquercitrin inhibits the progression of human bladder cancer	Chen et al. (2016b)
Isorhamnetin	Colon cancer	HT-29 and $Caco_2$	Isorhamnetin glycosides induced a higher percentage of apoptosis in HT-29 than in $Caco_2$, whereas isorhamnetin was more apoptotic in $Caco_2$	Antunes-Ricardo et al. (2014)
Kaempferide	Cervical cancer	HeLa (cervical), MDA-MB-231 (breast), HCT 116 (colon), and HL60 (leukemia)	Kaempferide induced apoptosis in cervical cancer cells	Nath et al. (2015)
Kaempferol	Atherosclerosis	HUVECs	Kaempferol alleviated ox-LDL-induced cell apoptosis	Che et al. (2017)

(continued)

Table 5 (continued)

Components	Chronic disease	Human/cell model	Key outcomes	References
	Human cancer	Human breast carcinoma (MCF-7) cells, human stomach carcinoma (SGC-7901) cells, human cervical carcinoma (Hela) cells, and human lung carcinoma (A549) cells	Kaempferol showed antiproliferative activity on a panel of human cancer cell lines	Liao et al. (2016)
Morin	Kidney injury	HK-2 cells	Morin acted as an ER stress inhibitor	Mo et al. (2019)
Myricetin	Colon cancer	HCT-15	Myricetin treatment reduced cell proliferation and induced apoptotic death of HCT-15 human colon cancer cells via BAX/BCL2-dependent pathway	Kim et al. (2014)
Quercetin	Blood pressure	Meta-analysis of randomized controlled trials ($n = 587$)	Quercetin significantly reduced blood pressure	Serban et al. (2016)
Quercitrin	Periodontal disease	hGF, hMSC	Quercitrin increased both soft and hard tissue regeneration of the periodontium	Gómez-Florit et al. (2015)
Rhamnazin	Inflammation	RAW264.7 cell	Rhamnazin was protective against LPS-induced cytotoxicity in macrophage cells	Kim (2016)
Robinin	Ox-LDL and inflammatory stress	Peripheral blood mononuclear cells were isolated from healthy human volunteers	Robinin ameliorates oxLDL-induced inflammatory insult through TLR4/NF-κB pathway	Janeesh et al. (2014)
Troxerutin	Acute cerebral infarction	Acute cerebral infarction patients ($n = 456$)	Troxerutin could improve neurological defects and promote functional recovery	Liang et al. (2017)

its clinical application. Zhou et al. (2019) investigated the protective effect and possible mechanism of icariin on cisplatin-induced nephrotoxicity on HEK-293 cells. They observed that icariin prevented cisplatin-induced HEK-293 cell injury via regulating NF-κB and PI3K/Akt signaling pathways. Chen et al. (2016b) reported that isoquercitrin inhibited bladder cancer via regulation of the PI3K/Akt and PKC signaling pathways. Antunes-Ricardo et al. (2014) showed that isorhamnetin glycosides showed cytotoxic effect against colon cancer cells, but their activity was affected by glycosylation. Nath et al. (2015), furthermore, demonstrated that kaempferide induced apoptosis in cervical cancer cells through activation of the caspase cascade.

Chronic obstructive pulmonary disease is another chronic inflammatory lung disease and is predicted to be the third leading cause of death worldwide by 2030. Lee et al. (2018) suggested that fisetin is a good drug candidate for improving the lung function of patients with chronic obstructive pulmonary disease, suppressing the TNF-α/NF-κB signaling pathway. Liu et al. (2017) observed that galangin extenuated osteosarcoma cells proliferation via selective activation of the transforming growth factor (TGF)-β1/Smad2/3 signaling pathway. Bipolar disorder is a common and severe psychiatric illness. Xiao et al. (2016) after investigating the effects of icariin on comorbid bipolar and alcohol use disorder in humans reported that icariin may decrease depressive symptoms and reduce alcohol consumption in persons with bipolar disorder and alcohol use. Atherosclerosis is a common disease in which plaque builds up inside your arteries. In addition, endothelial cells, macrophages, and smooth muscle cells have been demonstrated as the main cell types participating in atherosclerosis (Che et al. 2017). Che et al. (2017) observed that kaempferol abated ox-LDL-induced cell apoptosis by upregulation of autophagy via inhibiting PI3K/Akt/mTOR pathway in human endothelial cells. Endoplasmic reticulum (ER) stress may cause various kidney diseases. In a recent study, Mo et al. (2019) found that morin acted as an antioxidant and ameliorated ER-induced cytotoxicity in HK-2 cells. Rhamnazin was protective against LPS-induced cytotoxicity in macrophage cells via modulation of reactive oxygen species/reactive nitrogen species (Kim 2016). In short, dietary flavonols and *O*-glycosides are good candidates to prevent chronic diseases due to their pharmacological properties.

3.6 Application in Food

Food processing often causes losses in bioactive compounds, due to oxidation, enzymatic action, removal of skin or seeds, and leaching into water or oil that is then discarded (Rickman et al. 2007). On the other hand, the concentration of simpler derivatives may increase upon the breakdown of larger molecules. In the literature, there are several reports on the changes of contents of the flavonols, in particular quercetin, rutin, and kaempferol, due to different processing methods (Dos Reis et al. 2015; Nayak et al. 2015; Kamiloglu et al. 2016). A systematic review (Rothwell et al. 2015) collected extensive data and expressed the results as retention

factors (RFs), fold changes in flavonol content due to processing. According to this study, kaempferol and quercetin monoglycosides from broccoli were lost significantly as a result of boiling and frying (RF < 0.3); however steaming caused milder loss (RF $= 0.64$). Steaming also resulted in fewer losses of quercetin derivatives from carrot compared to boiling (RF $= 0.89$ and 0.37, respectively). On the other hand, similar losses of quercetin derivatives from onion were observed upon blanching, boiling, frying, and microwaving (RF $= 0.42$–0.54). Blanched and steamed cabbage and cauliflower contained increased amount of free quercetin; however free quercetin often represents only a small amount of all quercetin derivatives. Although different cooking methods caused a variation in the loss of rutin content in peeled potatoes (RF $= 0.39$–0.54), rutin content in carrots was affected similarly by boiling and steaming. On the contrary, almost all rutin was lost in cauliflower upon boiling, whereas steaming induced no significant change (RF $= 0.08$ and 0.76, respectively) (Rothwell et al. 2015).

In addition to food processing effect, interaction of flavonols with other components in the diet is also critical. Both macroconstituents, i.e., carbohydrates, lipids, and proteins, and microconstituents, i.e., minerals and vitamins, most often occur in foods in combination in a food matrix and are consumed together, which has an impact on the bioavailability of flavonols (Singh and Gallier 2014). Accordingly, a study carried out with rats (Matsumoto et al. 2007) reported that difructose anhydride III, i.e., an indigestible saccharide, promotes the absorption of αG-rutin, a soluble flavonol glycoside. The mechanism behind this observation may be the inhibition of αG-rutin conversion to rutin, a hardly absorbable compound due to its insolubility, by difructose anhydride III (Matsumoto et al. 2007). Another study assessing the effect of pectin on plasma quercetin levels and the fecal flora in mice supplemented with rutin found that the plasma quercetin and isorhamnetin concentrations were significantly higher in the pectin-rutin diet group compared to cellulose-rutin diet group. The authors suggested that pectin might increase the bioavailability of quercetin from rutin by changing the metabolic activity of the intestinal flora and/or the physiological function of the gut. Another explanation is that soluble fiber, e.g., pectin, could increase the gastrointestinal transit time, improving the absorption of flavonols (Tamura et al. 2007). In the study of Azuma et al. (2002), the effects of lipids and emulsifiers on the absorption of orally administered quercetin were investigated in rats. Co-administration of lipids such as lecithin and soybean oil or emulsifiers including sucrose fatty acid ester, polyglycerol fatty acid ester, and sodium taurocholate with quercetin had statistically no significant effects on absorption of quercetin, whereas, the combination of lipids and emulsifiers significantly enhanced the absorption of quercetin (Azuma et al. 2002). The same research group (Azuma et al. 2003) also examined the effects of co-ingestion of quercetin glucosides from onion with different sources of lipids including soybean oil, fish oil, beef tallow, and lecithin. The diet containing soybean oil significantly enhanced the accumulation of quercetin metabolites in plasma of rats. Fish oil and beef tallow also increased the plasma concentration to a similar extent to that with soybean oil, whereas lecithin was the most effective among all the lipids. Moreover, emulsifiers also showed an enhancing effect on the accumulation

of quercetin metabolites (Azuma et al. 2003). Later, another research group (Lesser et al. 2004) investigated the influence of dietary fat on oral bioavailability of quercetin in pigs. Quercetin was administered either as aglycone or as quercetin 3-*O*-glucoside in test meals differing in fat content (3, 17, or 32%). The results revealed that regardless of the chemical form applied, quercetin bioavailability was higher in the 17% fat diet compared to the 3% fat diet ($p < 0.05$) and no additional effect on bioavailability was observed when the flavonols were administered with diets containing 32% fat (Lesser et al. 2004). Same group further investigated the influence of fatty acid pattern of dietary fats on the oral bioavailability of quercetin, which was enhanced after intake of medium-chain and long-chain triacylglycerols, compared to the standard diet (Lesser et al. 2006). In a more recent in vivo study (Guo et al. 2013), overweight men and postmenopausal women ingested quercetin with a fat-free, low-fat, or high-fat diet. During high-fat diet, plasma quercetin maximum concentration and area under curve increased, compared to fat-free diet. Isorhamnetin and tamarixetin, the methylated metabolites of quercetin, were also increased during high-fat diet. The authors reported that it is likely that dietary lipids enhanced the micellarization of quercetin in the small intestine, which favored its solubility and absorption (Guo et al. 2013). In another study, healthy volunteers consumed supplements containing quercetin and kaempferol glycosides together with black tea, black tea with milk, green tea, and water. The addition of milk to black tea did not affect the plasma concentration of quercetin or kaempferol, and hence flavonols from tea were absorbed, and their bioavailability was not affected by the addition of milk (Hollman et al. 2001). Moreover, in the study of Ferri et al. (2015), effect of co-administration of α-tocopherol with quercetin and rutin is evaluated using a rat model. The concentrations of flavanols in plasma and brain indicated that α-tocopherol was able to promote quercetin and rutin transport. The authors indicated that the potential mechanism of enhanced transport of quercetin, rutin, and their putative metabolites might be due to the P-glycoprotein action and/or impairment of the phosphorylation/dephosphorylation mechanism, which controls the in-/outflux of metabolites across the blood-brain barrier (Ferri et al. 2015).

Besides the macro- and micronutrients given above, presence of other flavonoids can also affect the bioavailability of flavonols. For instance, Silberberg et al. (2005) performed a study on rats adapted to diets containing quercetin, or (+)-catechin, or both. When quercetin and (+)-catechin were fed together, their respective plasma concentration significantly decreased, whereas the urinary and hepatic concentrations were only reduced in case of quercetin. On the other hand, co-administration of quercetin and (+)-catechin had no effect on the formation of their metabolites, i.e., glucurono- and sulfo-conjugates (Silberberg et al. 2005). Similarly, Orrego-Lagarón et al. (2016) studied the simultaneous administration of naringenin and quercetin, which are common constituents of tomatoes and other fruits and vegetables, using an in situ model of intestinal perfusion in mice. The results showed that when naringenin and quercetin are administered together, the permeability coefficient values were decreased, whereas the levels of phase II metabolites were increased (Orrego-Lagarón et al. 2016).

3.7 Safety: Toxicity and Side Effects

Majority of studies on safety of flavonols are performed with quercetin. Several scientific bodies and national authorities declared information about the safety and recommended doses of quercetin. International Agency for Research on Cancer, the specialized cancer research agency of World Health Organization (WHO), declared that quercetin is not classifiable as to its carcinogenicity to humans. The Food and Drug Administration (FDA), the federal agency of the US Department of Health and Human Services, also approved quercetin as Generally Recognized As Safe (GRAS) under the intended conditions of use (Ożarowski et al. 2018). The average daily intake of quercetin was estimated as 200 mg and of approximately 460 mg for high consumers. Similarly, in Italy, based on a regulation, the amount of quercetin aglycone in dietary supplements is restricted to 200 mg per day. On the other hand, in Canada, 1200 mg quercetin was defined as the daily limit. However, in case of daily administration of 40–1200 mg quercetin, it has to be separated into two or three doses and consumed together with the meal. Furthermore, for uses over 12 weeks or during pregnancy and breastfeeding, the consumer should consult to a healthcare practitioner (Andres et al. 2018).

Several clinical trials showed that oral intake of quercetin in humans rarely results in adverse effects. Yet, no adverse incidences were reported in human intervention studies, in which volunteers were given quercetin at daily doses of 500 mg for 4–8 weeks (Javadi et al. 2014; Shi and Williamson 2016), 730 mg for 4 weeks (Edwards et al. 2007), or 1000 mg for 5 days, for at least 2 weeks or for 12 weeks (Ganio et al. 2010; Rezvan et al. 2017). On the other hand, in a study conducted with chronic pelvic pain syndrome patients for 1 month, one individual experienced headaches after taking a 1000 mg daily doses of quercetin for a few days. In addition, another patient complained about mild tingling sensation after each quercetin dose (Shoskes et al. 1999). In another study, in which the patients suffering from chronic hepatitis C were treated with daily doses of 250–5000 mg quercetin for 4 weeks, some patients developed mild stomach discomfort when quercetin was taken without a meal (Lu et al. 2016). Information on the safety of quercetin based on clinical trials is limited as there is no data considering long-term treatments ($>$12 weeks) with high dose of quercetin ($\geq$1000 mg per day) (Andres et al. 2018).

Animal studies showed that under certain conditions, quercetin might cause some implications including organ toxicity, cancer, and effects on endocrine system. According to chronic toxicity studies carried out with rats, consumption of approximately 1900–2100 mg quercetin per kg body weight per day resulted in reduced body weight, elevated organ weight (including liver and kidney), increased incidence of hyperplastic polyposis syndrome, presence of calcium oxalate crystals in urine, and existence of yellow-brown pigmentation in the stomach and small intestine (Ito et al. 1989; Dunnick and Hailey 1992; Program 1992). Furthermore, studies using different carcinogens showed that in rodents treated with 150–3400 mg quercetin per kg body weight per day, tumor development was observed in kidney (Zhu and Liehr 1994), colon (Pereira et al. 1996), pancreas (Barotto et al. 1998; Valentich et al. 2006), duodenum (Matsukawa et al. 2002), and mammary glands

(Singh et al. 2010). The possible mechanism behind the carcinogenic effect of quercetin was explained by the inhibition of catechol-*O*-methyltransferase enzyme resulting in increased formation of 4-hydroxyestradiol, which is a carcinogenic metabolite, as well as decreased formation of anti-carcinogenic metabolite 2-methoxyestradiol (Zhu and Liehr 1994; Singh et al. 2010). In addition, in male rats administration of 50–150 mg quercetin per kg body weight per day for 10 days increased the testosterone concentrations in the serum (Ma et al. 2004). In another study, Abd-Ellah et al. (2016) also demonstrated increased serum testosterone levels in male rats fed with 90 mg quercetin per kg body weight per day for 16 days. However, as some other studies conducted for longer periods did not report any effect of quercetin on serum testosterone levels in male rats, it is assumed that this increase in testosterone concentrations in the serum might be considered as a temporary effect of quercetin (Andres et al. 2018).

In addition to the above, both human and animal studies demonstrated that quercetin may interact with drugs and hence modulate their bioavailability. In humans, single or multiple administration of 300–1500 mg quercetin per day decreased the bioavailability of midazolam (sedative) (Duan et al. 2012; Nguyen et al. 2015) and talinolol (antihypertensive drug) (Wang et al. 2013; Nguyen et al. 2014), whereas enhanced bioavailability of cyclosporine (immunosuppressant drug) (Choi et al. 2004), fexofenadine (antihistamine drug) (Kim et al. 2009), and pravastatin (cholesterol-lowering drug) (Wu et al. 2012) was observed. Similarly, in some studies with rats, rabbits, and pigs, animals were fed with 0.6–100 mg quercetin per kg body weight per day for several days, and as a result, the bioavailabilities of many drugs including irinotecan, etoposide, tamoxifen, paclitaxel, doxorubicin (anticancer drugs), digoxin (heart failure drug), verapamil, diltiazem (hypertension, angina pectoris, and heart arrhythmia drugs), valsartan (antihypertensive drug), ranolazine (angina pectoris drug), and paracetamol were enhanced (Ożarowski et al. 2018). On the other hand, the bioavailability of simvastatin (cholesterol-lowering drug) and cyclosporine was reduced when taken together with quercetin (Hsiu et al. 2002; Cermak et al. 2009; Yu et al. 2011). Increased bioavailability of drugs may cause enhanced effectiveness of the drug; however it may also give rise to the potential side effects. In case of a possibility of an increased side effect, the dosage of drug should be adjusted (Andres et al. 2018).

Besides quercetin, kaempferol is also reported to possess some adverse effects including mutagenic and genotoxic effects (Galati and O'Brien 2004; Elgorashi et al. 2008). In addition, it has been reported that the consumption of kaempferol reduces the bioavailability of iron and/or folic acid and hence may cause some undesirable effects in iron and/or folic acid deficient patients (Lemos et al. 2007; Chen and Chen 2013). On the other hand, despite numerous in vitro studies on carcinogenic effects of kaempferol, there are no data from in vivo studies evidencing this effect (Devi et al. 2015). Similarly, for another flavonol, myricetin and its glycoside myricitrin, acute studies in mice did not provide evidence of genotoxicity, supporting the opinion of WHO's expert committee on food additives (JECFA) that myricitrin poses no safety concern for humans when consumed at current estimated dietary exposures (Hobbs et al. 2015).

3.8 Marketed Products

Nowadays, flavonol supplements, in particular quercetin supplements, are widely available in markets for affordable prices. The labels of these marketed products contain some health claims including support of immune and skin health, anti-inflammatory responses, cardiovascular and cholesterol health, and reduction of symptoms of arthritis. Additionally, these marketed products may help fight against allergies and pain; support circulation, mood, and energy levels; and help protect the kidneys, brain, and liver. The scientific studies related to these health claims of flavonols are given in detail in the previous sections. In some marketed products, flavonols are combined with other compounds to increase their low bioavailability. For example, there are a few quercetin supplements enhanced with 20–50% bromelain, which is an enzyme derived from the stems of pineapples. Although quercetin supplements enhanced with bromelain may provide increased absorption of quercetin, these products may not be suitable for those with pineapple allergies. In another product, quercetin is combined with vitamin C to ease the gastrointestinal discomfort that the consumed may experience and also to increase the antioxidant activity. Many of the products available on the market contain 500 mg of flavonol on average; however products with less or more amount of flavonols are also available.

3.9 Patents

In recent years, numerous patents have been published as a result of inventions related to applications of flavonols, in particular quercetin and its *O*-glycosides. These patents include methods of extraction of flavonols from natural sources (Zhang 2016; Cao et al. 2018; Shi et al. 2018a), production of nanocapsules (Wang et al. 2015; Krolevets 2016; Zhang et al. 2019), and prevention and treatment of various diseases including immune diseases (Park et al. 2017), liver disease (Kim et al. 2018), breast cancer (Lee 2018), and many others.

In Table 6, some recent US patents on health promoting effects of flavonols are presented. Mbikay et al. (2015) claimed that a therapeutically effective amount of quercetin-3-*O*-β-D-glucoside and, optionally, a therapeutically effective amount of a statin reduce the plasma cholesterol levels of patients. In another study, it has been suggested that quercetin combined with one or more of vitamin B3, vitamin C, and folic acid can be used to treat cancer together with a chemotherapy agent (Lines 2015). Same researcher also used a similar formulation, i.e., quercetin, vitamin B, vitamin C, and *Bauhinia forficata* extract, to treat metabolic syndrome and diabetes (Lines 2016). Moreover, a composition containing quercetin or isoquercetin, one or more of vitamin B3, vitamin C, and a folate, has been used to treat Zika virus infection. This formulation may also prevent microcephaly and treat and prevent infections of other *Flaviviridae* viruses (Lines 2017). Similarly, Bakar (2016) also claimed that quercetin, or analogues, or derivatives thereof have antiviral activity for prophylaxis or treatment of flavivirus infection or a disease resulting therefrom in humans or animals. The antiviral activity included the inhibition of virus attachment

Table 6 Recent US patents on health promoting effects of flavonols

Patent no	Date	Inventors (country)	Title	Major claims
US 2015/0190369 A1	Jul. 9, 2015	Mbikay et al. (Canada)	Quercetin-3-glucoside and uses thereof	Quercetin-3-*O*-β-D-glucoside (Q3G) increases the amount of cell surface low-density lipoprotein receptor (LDLR) on a cell and reduces the amount of functional protein convertase subtilisin/kexin type 9 (PCSK9) secreted by the cell Plasma cholesterol levels are reduced in patients treated with a therapeutically effective amount of Q3G and, optionally, a therapeutically effective amount of a statin
US 2015/0283112 A1	Oct. 8, 2015	Kim et al. (Korea)	Composition comprising myricetin as active ingredient for enhancing exercise performance or fatigue recovery	Myricetin or a pharmaceutically available salt thereof increases exercise capacity and enhances physical strength. Energy efficiency is increased by improving the function of mitochondria The composition comprising myricetin as active ingredient prevents aging and recovers from fatigue The composition also has an anti-obesity effect by increasing energy consumption
US 2015/0366838 A1	Dec. 24, 2015	Lines (Switzerland)	Method for treating cancer with a combination of quercetin and a chemotherapy agent	Combination of a chemotherapy agent and a composition that includes quercetin, one or more of vitamin B3, vitamin C, and folic acid may be used to treat cancer
US 2016/0000749 A1	Jan. 7, 2016	Bakar (Malaysia)	Method of inhibiting or treating a dengue virus infection with quercetin	Quercetin, or analogues, or derivatives thereof have antiviral activity for prophylaxis or treatment of *flavivirus* infection (that may comprise dengue virus types 1, 2, 3, and 4) or a disease resulting therefrom in humans or animals. The antiviral activity includes the inhibition of virus attachment to host cells and inhibition of intracellular virus replication

(continued)

Table 6 (continued)

Patent no	Date	Inventors (country)	Title	Major claims
US 2016/0129064 A1	May 12, 2016	Lines (Switzerland)	Method for treating metabolic syndrome and diabetes using quercetin and *Bauhinia forficata* extract	Composition containing quercetin, vitamin B, vitamin C, and *Bauhinia forficata* extract may be used to treat metabolic syndrome and diabetes
US 2016/0317442 A1	Nov. 3, 2016	Dajas et al. (Uruguay)	Nanosomal preparation of the complex formed by quercetin (or another flavonol, flavone, or a derivative thereof) and 2-hydroxypropyl-beta-cyclodextrin for intravenous use in cerebral pathological conditions	Cholesterol lecithin nanosomes, without propylene glycol, from the complex formed by quercetin (or another flavonol or flavone or a derivative thereof) and 2-hydroxypropyl-β-cyclodextrin, by means of a process that allows the safe, effective intravenous use thereof in the treatment of cerebral pathological conditions in adults and newborns The preparation stabilizes altered hemodynamic parameters in severe neonatal hypoxia in newborn pigs and is effective in protecting cerebral function in experimental Parkinson's disease models and in newborn pigs subject to hypoxia
US 2017/0007632 A1	Jan. 12, 2017	Lai and Lai (Taiwan)	Method for alleviating radiation injury with isorhamnetin-3-*O*-β-D-glucoside	Administration of a composition containing isorhamnetin-3-*O*-β-D-glucoside to a subject in need thereof alleviates the radiation injury
US 2017/0042924 A1	Feb. 16, 2017	Otsuka et al. (Japan)	Muscle atrophy inhibitor containing quercetin glycoside	Quercetin glycosides, which are safely ingestible for a long time, have inhibitory activity on the expression of myostatin involved in muscle atrophy
US 2017/0196834 A1	Jul. 13, 2017	Bei and Guo (China)	Preparation and application of flavonol as brain-targeting synergist	Kaempferide, rutin, troxerutin, myricetin, and hydroxy derivatives thereof, in particular their glycoside, ester, ether derivatives, can promote the drug molecules that have therapeutic or healthcare effect, such as ginsenoside, stilbene glucoside, resveratrol, levodopa, edaravone, vinpocetine, nicergoline, citicoline, oxiracetam, to enter brain tissues, to dramatically

(continued)

Table 6 (continued)

Patent no	Date	Inventors (country)	Title	Major claims
				enhance drugs concentrations in the brain tissues and effectively enhance the efficacy of drugs without increasing the plasma concentration
US 2017/0216246 A1	Aug. 3, 2017	Lines (Switzerland)	Method for treating Zika virus infection with quercetin-containing compositions	Composition containing quercetin or isoquercetin, one or more of vitamin B3, vitamin C, and a folate may be used to treat Zika virus infection. Composition may also prevent microcephaly and treat and prevent infections of other *Flaviviridae* viruses

to host cells and inhibition of intracellular virus replication. According to Otsuka et al. (2017), quercetin glycosides have inhibitory activity on the expression of myostatin involved in muscle atrophy. Likewise, another flavonol, myricetin or a pharmaceutically available salt thereof, has been shown to increase the exercise capacity and to enhance the physical strength. Increase in energy efficiency was due to the improved function of mitochondria. Myricetin is also claimed to prevent aging and recover from fatigue and has an anti-obesity effect by increasing energy consumption (Kim et al. 2015). In another study, nanosomal preparation of the complex formed with quercetin and 2-hydroxypropyl-beta-cyclodextrin was used in the treatment of cerebral pathological conditions in adults and newborns. The preparation was effective in protecting cerebral function in experimental Parkinson's disease models and in newborn pigs subject to hypoxia (Dajas et al. 2016). Similarly, kaempferide, rutin, troxerutin, myricetin, and hydroxy derivatives thereof, in particular their glycoside, ester, and ether derivatives, can promote the drug molecules that have therapeutic or healthcare effect, such as ginsenoside, stilbene glucoside, resveratrol, levodopa, edaravone, vinpocetine, nicergoline, citicoline, and oxiracetam, to enter brain tissues, to dramatically enhance drugs concentrations in the brain tissues and effectively enhance the efficacy of drugs without increasing the plasma concentration (Bei and Guo 2017). In addition to the above, administration of a composition containing isorhamnetin-3-*O*-β-D-glucoside to a subject in need thereof alleviates the radiation injury (Lai and Lai 2017).

3.10 Perspectives

In addition to the research already performed in the literature, new strategies need to be addressed to gain additional information. Below, a number of important points are highlighted as to how, in the future, one might approach to this topic differently, in order to maximize the knowledge gained from this chapter.

- Recently, the role of interindividual variability on the impact of flavonols on bioefficacy attracted great attention. So far, studies investigating this effect could not correlate the observed bioactivity with the bioavailability due to lack of data (Menezes et al. 2017). Hence, future studies, which include the parent compound and known metabolites, together with details of the individuals, i.e., age, gender, genotype, composition of gut microbiota, diet, lifestyle, and health status, are needed to address the effect of interindividual variation on bioavailability of flavonols and *O*-glycosides (Almeida et al. 2018).
- Encapsulation technology can be used to enhance the bioavailability of flavonols or to achieve controlled release of these compounds during digestion. Spray drying, coacervation, liposome entrapment, inclusion complexation, cocrystallization, nanoencapsulation, freeze-drying, yeast encapsulation, and nanoemulsion are some of the current technologies that are used to encapsulate bioactive compounds (Fang and Bhandari 2010). Although there are several reports in the literature that studied the encapsulation of flavonols (Hao et al. 2017; Azzi et al. 2018), the effect of this processing technique on the human body is not fully established yet. Therefore, further research on this topic is necessary.
- One of the factors affecting the bioavailability of flavonols is their interaction with other components in the diet. Co-digestion of flavonols with other macro- and microconstituents in foods will affect their bioavailability. In general, while indigestible carbohydrates, e.g., dietary fiber, proteins, and minerals, are likely to cause unfavorable effects on flavonol bioavailability, digestible carbohydrates, lipids, vitamins, and some other micronutrients, e.g., other flavonoids, alkaloids, and carotenoids, may enhance the bioavailability of flavonols. Interaction between flavonols and food matrix is a complex phenomenon that should be further investigated to provide maximum beneficial health effects to the consumers.
- Scientific information on the safety evaluation of flavonols from clinical trials is limited due to lack of relevant safety data, especially considering high-dose flavonol treatments for longer terms. Therefore, in future human intervention studies, it is important to investigate the safety and possible side effects of flavonols considering long-term treatments with high dose of flavonols (e.g., for quercetin >12 weeks and ≥ 1000 mg per day).

3.11 Conclusions

The focus of this chapter was the bioactive constituents, bioavailability, metabolism, bioactivity, benefits, food applications, safety issues, marketed products, and patents of dietary flavonols and *O*-glycosides. Flavonols, e.g., quercetin, kaempferol, myricetin, and isorhamnetin, most commonly occur as *O*-glycosides in dietary sources. In the human body, flavonol glycosides are cleaved to their aglycones and further metabolized to their glucuronidated, sulfated, and methylated conjugates. Moreover, compounds that reach the colon may be degraded by the colonic

microbiota to different metabolites, which may also contribute to the health beneficial effects of flavonol consumption with respect to cardiovascular diseases, diabetes, inflammation, viral infections, enhanced physical strength, and cancer prevention. Dietary intake of flavonols may be affected by the food processing, which often results in significant losses. Although rare adverse effects of flavonol consumption are reported, majority of the clinical trials and animal studies indicated that flavonol consumption is safe under the intended conditions of use.

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Chemopreventive Potential of Flavones, Flavonols, and their Glycosides

4

Randolph R. J. Arroo, Meng F. Wang, and Avninder S. Bhambra

Contents

Abstract

Epidemiological studies have long indicated a possible role for dietary flavonoids, notably flavones and flavonols, in the prevention of a range of degenerative diseases, e.g., cancer, diabetes, cardiovascular diseases, and neurological disorders like Parkinson's and Alzheimer's disease. The flavonoids are a large and variable group of compounds, comprising thousands of different structures. The bulk of the dietary flavonoids occur as glycosides.

The effect of flavonoid aglycones and their corresponding glycosides on cell metabolism and etiology of degenerative diseases has been a topic of interest for a number of decades. In contrast, the role of the metabolic products of dietary flavonoid that reach all parts of the human body through systemic circulation, has received much less attention.

Studies on animal and human metabolism have shown that the amount of flavone and flavonol glycosides which is absorbed intact is negligible; the bulk is absorbed only after deglycosylation. Thus, dietary glycosides are not likely to

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play a direct role in chemoprevention. However, the sugar groups on glycosides can greatly affect the bioavailability of flavones and flavonols. Flavonoids linked with indigestible sugars are not absorbed in the small intestine but are transported through the digestive tract to be degraded by gut bacteria in the large intestine.

The compounds that directly play a role in the prevention of degenerative diseases are most likely not dietary flavones themselves but rather their metabolites and conjugation products.

Keywords

Chemoprevention · Glucuronides; Methylated flavones · Quercetin

4.1 Introduction

Natural flavonoids, and their glycosides, are among the most abundantly available secondary metabolites in the human diet and, for several decades, now have been consistently linked with the prevention of a range of degenerative diseases (Imran et al. 2018; Smeriglio et al. 2016; Zhang and Tsao 2016). Though, any specific mechanisms of action for this group of phytochemicals are still a matter of debate (Khan et al. 2016).

The total amount of different naturally occurring flavonoids is estimated to be 10,000 (Dixon and Pasinetti 2010). Thousands of flavonoids that have been identified plants are discussed in a series of six triannual reviews of the flavonoid literature (Harborne and Williams 1995, 1998, 2001; Williams and Grayer 2004; Veitch and Grayer 2007, 2011). Initially, the main focus of attention was on anthocyanins – the intensely colored red to blue pigments of the flowering plants – and their potential role in plant ecology. In the last few decades, scientific interest shifted towards flavones and flavonols, due their possible beneficial effects as dietary components in the prevention of human cancer and other degenerative diseases. The presence of the more lipophilic flavonoids is regularly reported in extracts of dried powdered plant material, i.e., underground or aerial parts or even whole plants. Unfortunately, the precise location of lipophilic flavonoids in plants in many cases has remained unreported. Notably, the more highly O- and C-methylated and isoprenylated flavones and flavonols can be expected to reside in specific glands or trichomes on the surface of leaves, stems, or flowers rather than be evenly distributed throughout the tissues. In analysis of plant material, it would be good practice if surface flavonoids are removed by dipping the undamaged tissue in acetone, chloroform, or dichloromethane, after which the remaining polar flavonoids can be extracted with hot aqueous methanol thus providing two data sets for that tissue (Harborne and Williams 2001).

The formation of flavonoids in plants involves several biosynthetic pathways (Fig. 1). The aromatic B-ring is formed in the shikimate-phenylpropanoids pathway. The other aromatic ring (A) is formed via the polyketide pathway through C2 chain elongation of coumarate with malonyl-CoA as the condensing unit, initially resulting in the formation of the naringenin chalcone. The enzyme chalcone isomerase (EC

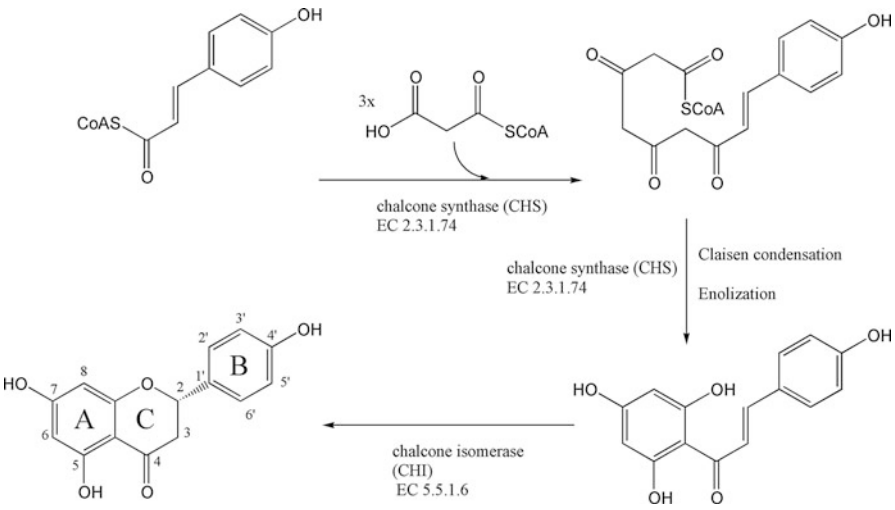

Fig. 1 Biosynthesis of flavonoids

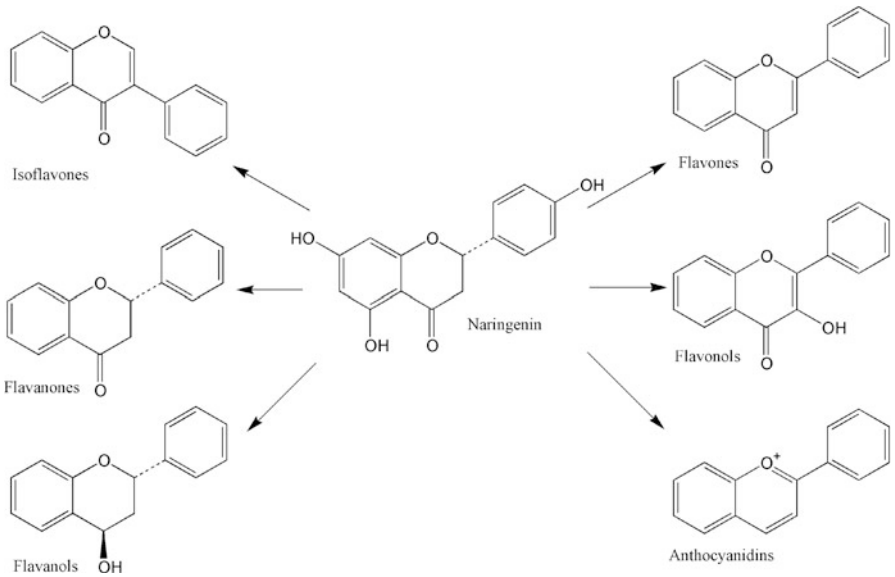

Fig. 2 Different classes of flavonoids with naringenin as common precursor

5.5.1.6), which is ubiquitously present in the plant kingdom, then catalyzes stereo-specific cyclization to form naringenin (Saito et al. 2013). The latter flavanone is the common precursor of all flavonoid types (Fig. 2). The sequence of events in the biosynthesis leading to naringenin explains why the most common substitution pattern in flavonoids involves *O*-substitutions at C-5, C-7, and C-4′. The wide

variety of structures is caused by further substitution patterns on the flavonoid skeleton. Predominant among these are examples characterized by simple patterns of *O*-substitution (OH, OMe) and prenylation (Veitch and Grayer 2007). Notably, *O*-methylation and prenylation result in compounds that are highly lipophilic (Harborne and Williams 1995, 1998). Advanced prenylation links the flavones with the terpenoid biosynthetic pathway and can result in a wide variety of structures, ranging from simple prenyl flavones to ring-closed furano, pyrano, chromano,

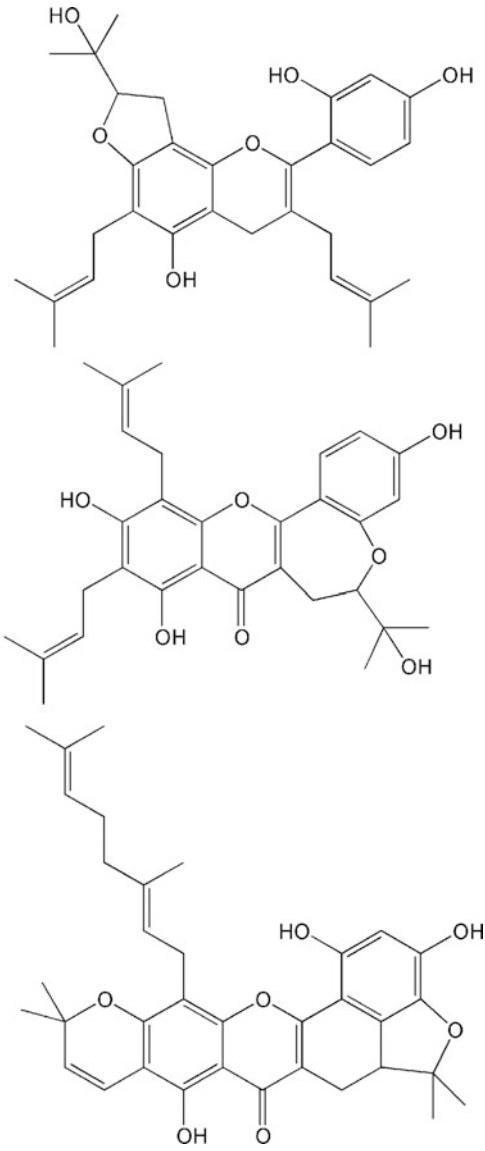

Fig. 3 Examples of highly prenylated flavonoids. (After Harborne and Williams 2001)

and methylenedioxy derivatives (Harborne and Williams 2001) in which the flavonoid core may be difficult to spot (Fig. 3).

A further cause for variation in the flavonoids is the formation of glycosides. The bulk of flavonoids in plants exist in glycosidic form, either as *O*-glycosides or, more rarely, as *C*-glycosides. The *O*-glycosides of flavones and flavonols, and the *C*-glycosides of flavones form the most numerous groups of glycosylated flavonoids (Veitch and Grayer 2011). The most common sites for *O*-glycosylation are C-7 on the flavones, and C-7 and C-3 on the flavonols; 5-*O*-glycosylation of flavones and flavonols is comparatively rare in nature (Harborne and Williams 1995). The sites for *C*-glycosylation on flavones are either C-6 or C8, and several di-*C*-glycosides have been reported (Jay et al. 2006; Xiao et al. 2016). A wide variety of sugars can be involved in glycosylation. Typically, sugars are all in the pyranose form and sugar linkages are β- for glucose, galactose, xylose and α- for rhamnose (Harborne and Williams 1998). Acylation of the sugars is common, with acetic acid as the most frequent acyl substituent, though other acids, e.g., malonic, p-coumaric, or caffeic acid, have been reported as acylating groups (Veitch and Grayer 2007, 2011). As a general rule, glycosylation makes the relatively lipohilic flavones and flavonols more water-soluble so that they are not necessarily restricted to glands or trichomes on the surface of leaves, stems, or flowers but may be more evenly distributed in plant tissue. Acylation converts water-soluble flavonoid glycosides back again into lipophilic substances and may be seen as a means to regulate distribution of this group of compounds within the plant tissues. The exact role of flavones and flavonols for the plant is still a matter of speculation, though it is generally assumed that they protect plant tissue against the damaging effects of UV-B radiation or accumulation of oxygen radicals which is part of the plant's defense system against fungal attack. Ionizing radiation and radical oxygen species have been considered a major cause of damage to macromolecules not only in plants but in humans too. The possibility that dietary flavones and flavonols may protect against this damage was proposed almost four decades ago (Ames 1983) and has been considered time and again. However, clinical trials so far have only provided weak correlations (Del Bo et al. 2019; Grosso et al. 2017; Jin et al. 2012; Ried et al. 2017; Suen et al. 2016; Tang et al. 2016).

4.2 Screening for Biological Activity

Papers that discuss the properties of flavonoids commonly start with a list of biological activities that are ascribed to this compound group. This is remarkable since neither the Food and Drug Administration (FDA) nor the European Food Safety Authority (EFSA) have recognized any dietary flavonoid as a drug, i.e., a product intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease (EFSA 2010; FDA 2017). However, a number of herbal products rich in flavonoids are listed in community herbal monographs and have received formal marketing authorization based on traditional medicinal uses of the herbs (European Parliament, Council of the European Union 2004).

Most of the biological activities ascribed to flavonoids are based on preclinical studies. The last few decades have seen remarkable developments in molecular biology, bioanalytical techniques, and handling of large data sets, and these developments have significantly altered the way preclinical studies are viewed.

The large anticancer drug discovery programs of the 1960s and 1970s were all based on phenotypic screening, i.e., assays to find compounds that improve a disease phenotype. Initial primary screening involved mice in vivo bearing transplantable leukemia cell lines L1210 or later replaced by panel of tumor xenografts in nude mice (DeVita and Chu 2008). These in vivo assays resulted in the development of a range of plant-derived anticancer drugs that are still in clinical use today, e.g., vincristine and vinblastine from *Catharanthus roseus* (syn. *Vinca rosea*), podophyllotoxin derivatives etoposide and teniposide from *Sinopodophyllum hexandrum* (syn. *Podophyllum emodi, Podophyllum hexandrum*), combretastatin A-4 from *Combretum caffrum*, taxanes (paclitaxel, docetaxel, cabazitaxel) from *Taxus* species, and campthothecins (topotecan, irinotecan) from *Camptotheca acuminata*. In the 1990s, cell culture systems became more sophisticated and the screening system was changed to a panel of 60 human cancer cell lines grown in vitro. The latter in vitro cell viability screening assay is still considered a form of phenotypic screening; the assay was used to determine cytotoxicity of compounds without initial concern about specific targets or mechanisms of action. However, the NCI-60 screen allowed identification of differential cytotoxicity fingerprints which may be used to assign compounds to specific mechanistic classes or even identify novel mechanistic classes (Boyd and Paull 1995).

Around the turn of the millennium, developments in molecular biology, notably the expansion of OMICS (genomics, transcriptomics, proteomics) and PCR-based, heralded the shift to functional screening as the primary tool in drug discovery. A better understanding of the molecular basis of cancer, but also of metabolic disorders like diabetes or neurodegenerative diseases like Parkinson's and Alzheimer disease, made the development of therapies targeted at specific proteins or transcription factors that play a key role in degenerative diseases an achievable and logical approach. The increasing amount of information genome databases, combined with improved PCR-based methods for gene isolation and cloning, allowed the production of substantial amounts of recombinant proteins that became available for detailed studies of protein-ligand interactions. This in turn opened the field of bioinformatics, where interactions of virtual model proteins with novel ligands is studied by docking experiments in silico. The discovery and subsequent development of a range of small-molecule kinase inhibitors is probably the best example of what can be achieved with a target-based approach to drug screening (Zhang et al. 2009).

Target-based screening proved particularly successful for identifying therapeutic biologics (e.g., enzymes, antibodies, peptides, vaccines) that function by interfering with a molecular activity and whose mechanism of action is dependent on a specific protein target. However, many of the small-molecule novel drug entities that may have looked promising in the initial functional screening assays did not do well in later clinical trials. Of the drugs that were approved by the FDA in the decade ranging from 1999 to 2008, an era in which the major focus was on target-based

approaches, the contribution of phenotypic screening to the discovery of first-in-class small-molecule drugs exceeded that of target-based approaches (Swinney and Anthony 2011).

Current screening of synthetic and natural product libraries for novel therapeutic small molecules usually applies a mixed approach, known as mechanism-informed phenotypic screening (Moffat et al. 2014; Coussens et al. 2017). The phenotypic screen is considered more physiologically relevant and less artificial than pure target-based assays because intact cells and native cellular environment are used (Zheng et al. 2013). Cells grown in a monolayer on plastic have remained the bedrock of cancer drug discovery assays, although three-dimensional growth of non-adherent cells is regarded as a more representative model. In addition, many disease models have been developed and applied to phenotypic compound screening, e.g., strains of *Caenorhabditis elegans*, zebrafish, *Xenopus laevis*, and *Drosophila melanogaster*. Then, rather than simply determining the effect of drugs on cell survival, molecular events such as gene expression and protein phosphorylation are monitored. Also, assays can be used that link complex cell signalling networks to transcriptional activation and expression of reporter genes (e.g., coding for luciferase or green fluorescent protein).

Many of the defined drug targets are single components in extended cell signalling networks, and their inhibition does not necessarily result in an altered phenotype. What was often ignored in pure target-based screening is that within cell signalling, a redundancy is common, and compensatory and feedback mechanisms can undo the effect of a drug (Moffat et al. 2014). In addition, the drugs often target part of the canonical pathways, defined in the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/). Less information is available on non-canonical pathways that may operate in aberrant cells that lay at the basis of many degenerative diseases. Regulation of cell metabolism occurs at many levels, e.g., too often mRNA expression is seen as a shorthand for protein activity whereas comparative OMIC studies have shown that transcriptomics or RNA-sequence data does not always correspond to proteomic data. In the same vein, when posttranslational processes can make that, there may be no correspondence between proteomics and actual protein/enzyme activity (Amoedo et al. 2017). The latest addition to the OMICS cluster is metabolomics, which allows identification of differences of metabolic profiles between different cell types of tissues. The method has been applied to identify novel targets tumors, but the absence of comprehensive metabolomics repositories still poses a limitation to what can be a powerful tool to monitor the rerouting of metabolic pathways in diseases processes (Goveia et al. 2016). Another limitation of metabolomics may be the limited spatial resolution, as cell metabolites can diffuse within tissues and even the entire organism, making it difficult to pinpoint any changes to specific cell types (Amoedo et al. 2017).

All the screening assay mentioned above have been applied to flavonoids, partly because these compounds are so ubiquitously present in a wide variety of medicinal herbs and in fruits and vegetables that are consistently linked with good health and prevention of degenerative diseases. In silico target-based screening, often followed up with in vitro inhibition assays, has indicated that many flavonoids can potentially

inhibit kinases (Hou and Kumamoto 2010; Zhao et al. 2019), androgen receptors (Singh et al. 2017), and ABCG2 multidrug transporters (Boumendjel et al. 2011), or can trigger anti-inflammatory pathways (Alfa and Arroo 2019).

4.3 Structure–Activity Relationship (SAR)

Series of screenings of flavonoids for biological activity has resulted in the identification of pharmacophores, though what exactly defines a flavonoid pharmacophore, i.e., the features necessary to ensure interactions with a specific biological target (Wermuth et al. 1998), depends on the type of screening.

When considering flavonoid-induced inhibition of multidrug resistance-associated protein (MRP) or of the breast cancer resistance protein (BCRP, part of the ABCG2 protein family), both energy-dependent drug-efflux pumps which normally export steroid molecules, the presence of the 4-carbonyl, the 5-hydroxyl group, the 3-hydroxyl group, and the 2–3 double bonds are required for the activity (Boumendjel et al. 2002; Scotti et al. 2012; Fang et al. 2019). Docking studies suggested that the hydrophobic B-ring would bind to the steroid-binding region whereas the 3-hydroxyl in combination with the 4-carbonyl mimics the adenine in ATP (Boumendjel et al. 2002).

Looking for cytotoxic effects against breast cancer cell lines or inhibitors of aromatase, the C-4 keto group of flavonoids was considered essential, the 7-hydroxyl enhanced the activity, and flavones show greater activities than flavanones, i.e., 2–3 double bond increases the efficacy. Glycosylation of flavones removed their activity (Manthey and Guthrie 2002; Li et al. 2007).

Considering antioxidant capacity and anticancer activity, the important features are 4′-hydroxyl group is essential, a second hydroxyl group in the B-ring resulting in a catechol substitution pattern enhances the activity, and a 3-hydroxyl group on the C-ring increases the antioxidant activity. Again, comparing flavanones and flavones showed that the 2–3 double bond conjugated to the 4-carbonyl group in ring C is essential (Pannala et al. 2001; Scotti et al. 2012).

Combined in vitro enzyme assays and in silico docking studies, assessing the interaction of flavones with glycogen synthase kinase-3β (GSK-3β), indicated that hydroxyl groups that form hydrogen bonding with the amino acid residues in the enzyme contribute to provide stability, while sugar conjugations at C-3 or C-7 are less suitable in providing stability (Johnson et al. 2011). Similar experiments, assessing the interaction between flavones and glyoxalase I (GLO I), a key enzyme in pathways leading to the detoxification of methylglyoxal, found that a plane configuration of *cis* C-4 ketone and C-5 hydroxy was essential, as this configuration mimics an enediolate intermediate that forms in the detoxification pathway of methylglyoxal. Hydroxy groups at the B-ring of flavones enhanced the inhibitory activity of the flavonoid compounds, whereas C-3 or C-7 glucuronic acid conjugates were found to be very poor inhibitors. In this model, the double bond between C-2 and C-3 did not significantly affect inhibitory effects (Takasawa et al. 2008).

The radical scavenging effects luteolin and its glycosides cymaroside (7-O-β-D-glucoside), cesioside (7-O-β-D-primeveroside), isoorientin (6-C-β-D-glucoside), and stereolensin (6-O-β-D-glucoside) were assayed, and their inhibition of pro-inflammatory pathways was tested in rat leukocytes. The presence of catechol substitution pattern on the B-ring and a 5-OH substitution of the A-ring were considered to significantly contribute to the anti-inflammatory and antioxidant activities of the flavone. Glycosyl substitutions at C-6 or C-7 did not affect the radical scavenging properties but slightly diminished the inhibitory effect on induction of pro-inflammatory pathways (Odontuya et al. 2005).

Overall, mechanism-informed phenotypic screening of flavonoids has provided the rough contours of a flavonoid pharmacophore with anti-inflammatory and potential cancer preventive properties (Fig. 4). The presence of a C-2,3 double bond and hydroxyl groups at C-5 and C-4′ are crucial for the cytostatic effects. Additional hydroxyls on the B-ring resulting in two hydroxy substituents *ortho* to each other tend to enhance the activity, whereas a methoxy group in the 3′-position on B-ring lowers the antiproliferative activity. Flavones tend to be more potent that flavonols (having a hydroxyl at C-3), but C-6 hydroxyl groups significantly decrease the cytotoxic activity (Plochmann et al. 2007; Menezes et al. 2016). A structural feature common in flavonols, i.e., 3-hydroxyl and 4-carbonyl groups, mimics the adenine 6-amino and 1-nitrogen groups, respectively in ATP, and thus allows interference with energy-dependent transporter proteins (Conseil et al. 1998; Barron et al. 2002).

Glycosylation, notably at C-3, generally diminishes the cytotoxic activity, whereas the formation of methylethers or prenylation, i.e., modifications that make the flavones more lipophilic, tend to increase the activity (Walle 2007; Menezes et al. 2016).

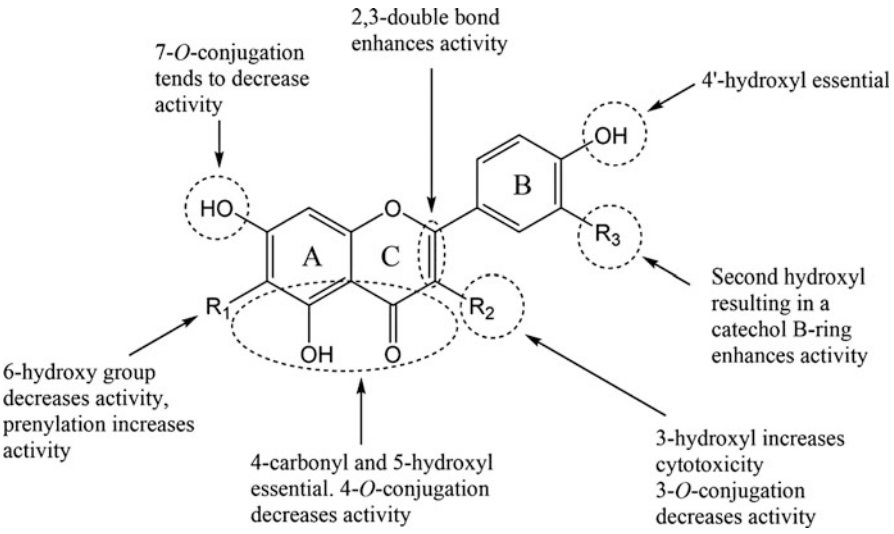

Fig. 4 Generalized flavone pharmacophore

4.4 In Vitro Activities

Generally, in vitro assays have shown that *O*-glycosylation reduces the bioactivity of flavones regarding prevention of degenerative diseases, e.g., antioxidant activity, antidiabetic activity, anti-inflammation activity, anticancer activity, anticoagulant activity, antiplatelet activity, aldehyde oxidase inhibition, and immunomodulatory activity. Though, some other types of bioactivity may be enhanced by *O*-glycosylation of flavones (Xiao 2017). However, the in vitro effects of dietary flavones and their glycosides are of limited practical use if we wish to understand the role of these compounds in the prevention of degenerative diseases. If we look at these compounds as drugs with a particular pharmacophore, what is important is that they can interact with their specific biological target (Wermuth et al. 1998). In other words, the flavones have to reach sufficiently high concentrations to elicit a therapeutic response. In in vitro assays, bioactivity is commonly expressed in IC_{50} for inhibition of physiological processes or EC_{50} for drugs that activate a process. Both IC_{50} and EC_{50} are measures of concentration, expressed in molar units. The concentration of flavones and flavone conjugates (glucuronides, sulfates) in human systemic circulation is less than 1 μmol/L (Hostetler et al. 2017). Thus, if any in vitro flavone bioactivity is reported with a value, expressed as IC_{50} or EC_{50}, of more than 1 μmol/L, then this compound is not likely to ever elicit a therapeutic response in vivo. Consequently, these compounds should be labelled as having "weak activity"; compounds with IC_{50} or EC_{50} values higher than 10 μM should be labelled as "inactive."

If we want to assess the health-enhancing role of dietary flavones and their glycosides, only in vitro bioactivity experiments are not sufficient. We need to assess the pharmacokinetics of the compounds and study their absorption, distribution, metabolism, and excretion (ADME).

The ADME properties determine the concentration of bioactive compounds in systemic circulation and also how long an effective concentration is maintained after a meal.

4.5 ADME Properties

Many detailed ADME studies focus specifically on quercetin and its glycosides, due to their abundant availability. Dietary quercetin occurs almost exclusively as β-glycosides, where the aglycone can be bound to a variety of sugars depending on the food source, e.g., apples are rich in galactosides, rhamnosides, and arabinosides, whereas in onions, glucosides are the main glycosidic form. The sugar moiety was shown to be an important determinant of the bioavailability of quercetin from foods (Arts et al. 2004). This may seem odd since studies in vitro using a Caco-2 model of intestinal absorption and in situ using a rat intestinal perfusion model have shown that quercetin is not absorbed as a glycoside, but mainly in its free aglycone form via passive diffusion in the small intestine (Guo and Bruno 2015). Lactase-phlorizin hydrolase (LPH), a mammalian β-glycosidase present in the brush border membrane

of the small intestine, is the main determinant of quercetin bioavailability. Absorption of quercetin glucosides is higher than that of the aglycone (Cermak et al. 2003), because the glucosides are more water-soluble than free quercetin and thus diffuse more easily through the intestinal tract to reach the brush border membrane where they then can be hydrolyzed and subsequently absorbed (Guo and Bruno 2015). However, this is true only for *gluco*sides; LPH efficiently hydrolyses quercetin-3-*O*-β-glucosides and 4′-*O*-β-glucosides, but not other glycosides like quercetin-3-*O*-β-galactoside, quercetin-3-*O*-β-rhamnoside, and quercetin-3-*O*-α-arabinopyranoside (Arts et al. 2004). The latter glycosides are not absorbed in the small intestine but pass into the large intestine where they are degraded by intestinal microflora. The initial degradation step is deglycosylation, mediated by gut microbiota-derived β-glucosidases, that generates quercetin aglycone some of which in turn can be absorbed from the large intestine through passive diffusion. The latter absorption step is much less efficient than that in the small intestine, due to the differences in surface area available for absorption in both organs. Flavone-*C*-glycosides are not hydrolyzed by LPH, and they too reach the large intestine unaltered where they are deglycosylated by gut microflora (Zhang et al. 2007; Xiao et al. 2016). Deglycosylation is only the first step in the degradation of flavones and flavonols by human gut microflora; the aglycone is further degraded.

The exact way that flavones and flavonols are degraded in the large intestine depends for a large part on the combination of the bacterial species that make up the microbiome in this organ. The microfloral profile differs from person to person and is shaped by genetic factors and environmental factors such as diet, lifestyle, geography, infection, and medication. For any one individual, the composition of the gut microbiome tends to be stable over time (Sen and Orešič 2019). Based on human intestinal bacterial species so far identified that are involved in the conversion of flavonoids, a general degradation pathway can be sketched out (Braune and Blaut 2016). The bacterial degradation of flavonol and flavone aglycones starts with the reduction of the C2-C3 double bond yielding the corresponding flavanonols and flavanones (Schoefer et al. 2003). The next step is C-ring fission, resulting in the formation of chalcones. The next steps differ as a result of the 3-hydroxyl group in flavonols, which is absent in flavones. The flavonol forms a chalcone intermediate that can undergo keto-enol tautomerism and is eventually converted into an aurone; the hydrolytic opening of the five-membered ring of the aurone results in the formation of phenylacetic acid derivative and phloroglucinol. The fission of the heterocyclic ring of flavones leads directly to a chalcone structure, which is reduced to the dihydrochalcone, and then hydrolyzed to form a phenylpropionic acid derivative and phloroglucinol. The phloroglucinols are further degraded into short-chain fatty acids (Brune and Schink 1992) (Fig. 5).

Very little, if any, of the flavone and flavonol glycosides that are present in the human diet reaches systemic circulation. Indeed, in vitro studies with rat jejunal tissue have shown that some quercetin-3-*O*-glucoside can be absorbed as a glycoside through sodium-dependent glucose transporter 1 (SGLT1) (Wolffram et al. 2002; Day et al. 2003), but at the same time, multidrug resistance protein (MRP2, aka ABCC2) actively transports 4′-*O*-glucoside and 3-*O*-glucoside out of the brush

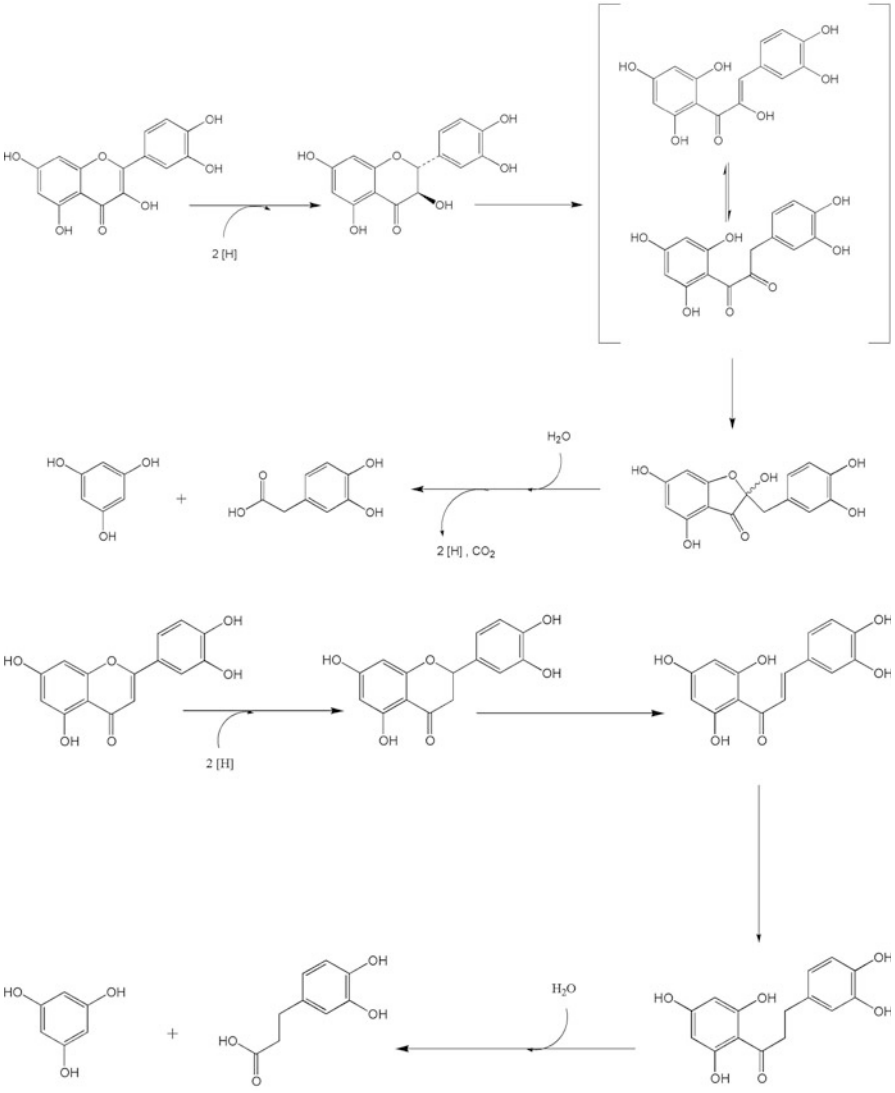

Fig. 5 Generalized scheme of bacterial degradation of flavonols (**a**) and flavones (**b**). (After Braune and Blaut 2016)

border cells and back into the lumen of the small intestine (Walgren et al. 2000; Luo et al. 2015), together with other flavone conjugates (O'Leary et al. 2003).

Flavonols and flavones that enter into the circulatory system are rapidly conjugated to form glucuronides, sulfates, or mixed sulfo-glucuronide conjugates; these reactions are catalyzed by human β-glucuronidases and sulfotransferases (Day et al. 2001; O'Leary et al. 2003; recently reviewed by Williamson et al. 2018; Wang et al. 2017). Quercetin is also a substrate for human catechol-*O*-

methyltransferase, resulting in the formation of 3′-methylquercetin (isorhamnetin) and 4′-methylquercetin (tamarixetin) (Cermak et al. 2003; O'Leary et al. 2003). These methylated derivatives can also be further converted into glucuronides and sulfates.

Considering that dietary flavone- and flavonol-glycosides do not enter into human systemic circulation, and that flavones and flavonols circulate in plasma not as aglycones, but predominantly as conjugated metabolites, it is the latter conjugates that ought to be the focus studies on the biological activity of flavonols in humans (Day et al. 2001; Spencer et al. 2004).

4.6 Further Screening for Biological Activity

It has been argued for decades that, if we wish to understand the role of dietary flavonoids, the transformations these compounds undergo during digestion and cellular metabolism should be analyzed, and the bioactivities of both unmetabolized parent compounds and their putative metabolites should be evaluated. However, literature on in vitro studies addressing this issue has remained scarce (Aragonès et al. 2017). Though many metabolites of flavones and flavonols have been identified, the challenge remains to get sufficient amounts of well-characterized purified material to perform bioactivity studies. Synthetic methods have been developed to obtain flavonol glucuronides and sulfates, compounds that are of interest as the main human plasma metabolites (Needs and Kroon 2006; Zhang et al. 2012).

Cell viability assays showed that quercetin, and its metabolites quercetin-3′-sulfate, quercetin-3-glucuronide, isorhamnetin, and isorhamnetin-3-glucuronide inhibit the growth of human breast cancer MCF-7 cells in a dose-dependent manner. Although the concentrations that were tested in the in vitro assay (25–100 μM) were at least an order of magnitude higher than would ever be found in vivo, neither of the metabolites affected cell viability of nonmalignant mammary epithelial cell line H184B5F5 M10 at doses up to 100 μM (Wu et al. 2018a, b). Luteolin-3′-*O*-, 4′-*O*-, and 7-*O*-glucuronides reduced the expression of inflammatory genes in lipopolysaccharide-treated RAW264.7 cells, a commonly used model for inflammation studies (Kure et al. 2016). In both studies, in vitro the aglycone showed stronger inhibitory activity than the conjugated metabolites, though since the concentration of these aglycones in vivo is almost negligible, it is the conjugated compounds that should be considered as the active pharmaceutical ingredients. Quercetin and its sulfated and methylated metabolites quercetin-3′-*O*-sulphate, isorhamnetin, and tamarixetin were all shown to inhibit the activity of xanthone oxidase, an enzyme that plays a key role in purine catabolism. The IC_{50} values found were less than 1 μM, which makes this type of activity a feasible option in vivo, e.g., for the treatment of gout (hyperuricemia). The metabolites quercetin-3-*O*-glucunoride and isorhamnetin-3-*O*-glucuronide did not inhibit xanthine oxidase (Mohos et al. 2019).

Some recent studies focused on the biological activity of the degradation products of flavonoids that are produced in the large intestine and then absorbed (Hanske et al. 2013). Using in vitro kinase assays, the flavonol degradation product

2,4,6-trihydroxybenzoic acid was demonstrated to inhibit CDKs 1, 2, and 4 in a dose-dependent manner. No such activity was found for 3,4,5-trihydroxybenzoic acid or phloroglucinol. Docking studies, in silico, identified putative key amino acids involved in these interactions. Again, the concentrations used in the in vitro bioassays (50 μM–1000 μM) were much higher than can ever be expected in an in vivo system (Sankaranarayanan et al. 2019). Colonic quercetin metabolites 4-methylcatechol, 3-(3,4-dihydroxyphenyl)propionic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and phloroglucinol were poor inhibitors of xanthine oxidase (Mohos et al. 2019). However, pyrogallol, a breakdown product of more dietary flavonoids, strongly inhibited xanthine oxidase activity ($IC_{50} = 1.8$ μM); this is relevant since sulfate conjugates of pyrogallol can reach plasma concentration higher than 10 μM (Pimpão et al. 2015).

It is often argued that, if we wish to understand the role of dietary flavonoids in the maintenance of health or in the prevention of degenerative diseases, the compounds need to be tested in vivo. Epidemiological studies and human intervention studies keep hinting at the potential of dietary flavonoids to prevent degenerative diseases (Potì et al. 2019; Rodríguez-García et al. 2019). Some stronger evidence comes from animal studies, e.g., dietary quercetin has been shown to affect gene expression and function of epididymal adipose tissue in Western diet-induced obese mice, and to ameliorate the effects of diet-induced metabolic syndrome (Kobori et al. 2016). However, epidemiological studies, human intervention studies, and in vivo animal studies only provide limited insight into the mechanism of chemoprevention. To fully elucidate the internal workings of the chemoprevention process, a reductionist methodology and in vitro mammalian and human cell cultures will remain an important tool for the foreseeable future. Whether dietary flavone and flavonol glycosides directly affect cell metabolism in vitro is a question of limited practical interest, because glycosides never reach the target cells in vivo. Glycosides do affect the bioavailability of dietary flavones and flavonols in vivo, but if we wish to understand how chemoprevention works, in vitro tests should best concentrate on the ways that flavone and flavonol metabolites and their conjugates affect cell metabolism.

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Flavonoid C-Glycosides in Diets

5

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Abstract

Flavonoids are one of the most widely occurring secondary plant constituents and are rich in vegetable and fruit diets as well as beverages of plant origin. In flavonoid glycosides, the sugars can either be linked to the aglycone via an ether bond (*O*-glycosides) or via a C–C- bond resulting in flavonoid C-glycosides. Their occurrence in food plants, their role in bioactivity of food, and their catabolism are covered in this chapter. The major class of C-glycosylflavonoids in food plants is represented by flavones, in addition dihydrochalcones and C-glycosylisoflavones can be found. Citrus fruits can be considered as a major source of C-glycosylflavones, whereas in most cases relatively low amounts have been found in cereals. A rich source of C-glycosylated dihydrochalcones are tomatoes, as well as rooibos and honeybush herbal teas, and the most common C-glycosylisoflavone puerarin is mainly consumed via kudzu roots. Due to their higher chemical stability in terms of hydrolysis during cooking and also after ingestion, they can be considered as a specific group within flavonoids. Their metabolic fate is clearly different from *O*-glycosidic flavonoids with absorption of intact glycosides, followed by phase II metabolization. However, also deglycosylation by gut microbiota and degradation of aglycones to compounds like (hydroxy)phenylpropionic acids have been recognized. Only limited data on the actual daily intake of C-glycosylflavonoids including information on content in fresh and processed food are available.

Keywords

C-Glycosylflavonoids · Flavonoid C-glycosides · Citrus fruit flavonoids · Cereal flavonoids · Flavonoid metabolisms · Flavonoid bioactivities · Flavones · Dihydrochalcones · Isoflavonoids

Abbreviations

ABTS	2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt
CGF	C-glycosylflavonoids
CGT	C-glycosyltransferase
COX	Cyclooxygenase
CYP	Cytochrome P450 enzymes
DPPH	2,2-diphenyl-1-picrylhydrazyl
ESI	Electrospray ionization
IC_{50}	Half maximal inhibitory concentration
IL	Interleukin
MCF	Human breast cancer cell line
MIC	Minimal inhibitory concentration

mRNA	Messenger RNA
OGF	*O*-glycosylflavonoids
PGE-2	Prostaglandin E2
P-gp	P-glycoprotein
PXR	Pregnane-X- receptor
RAW	Mouse macrophage cell line
TE	Trolox equivalents
TNF-α	Tumor necrosis factor α
Vero	African green monkey kidney cells

5.1 Introduction

Flavonoids are one of the most widely occurring secondary plant constituents and are rich in vegetable and fruit diets as well as beverages of plant origin. In plant food, flavonoids mostly occur as glycosides, i.e., bound to one or more monosaccharide moieties. Major structural diversity of flavonoids arises from hydroxylation, methoxylation, and glycosidation. The sugars can be attached to the aglycone via an ether bond (*O*-glycosides) or being directly linked to the aglycone via a C–C bond resulting in flavonoid C-glycosides (C-glycosylflavonoids, CGF). This type of glycosidation results in distinct features compared to *O*-glycosides concerning their resistance to hydrolysis. Hence, C-glycosylflavonoids represent a specific group of plant phenolics which is often underestimated. Their occurrence in food plants, their role in bioactivity of food, and their catabolism will be covered in this chapter.

5.2 Bioactive Constituents

5.2.1 Chemistry of Flavonoids with Emphasis on C-Glycosylflavonoids

Chemically, their basic structure can be designated as 2-phenylbenzopyrane which is deriving major flavonoid classes, i.e., flavones, flavonols, flavanones, flavanols (which are related to proanthocyanidins), chalcones including dihydrochalcones and α- and β-OH-dihydrochalcones, and anthocyanidins. Migration of the aromatic ring from C-2 to C-3 position of the basic skeleton leads to isoflavonoids. The nomenclature of flavonoid classes follows the oxidation pattern of the C-ring. Figure 1 illustrates the nomenclature of flavonoids and shows the basic structures of most important plant flavonoid classes.

In C-glycosylflavonoids, glucose is the predominant sugar moiety, although also xylose, arabinose, rhamnose, galactose, and apiose were identified as glycosidic moieties. Additionally, C-glycosylflavonoids can be acylated with acetyl, p-coumaroyl, sinapoyl, feruloyl, and/or vanilloyl residues. By far the majority of C-glycosylflavonoids in plant derived food can be found among flavones, more

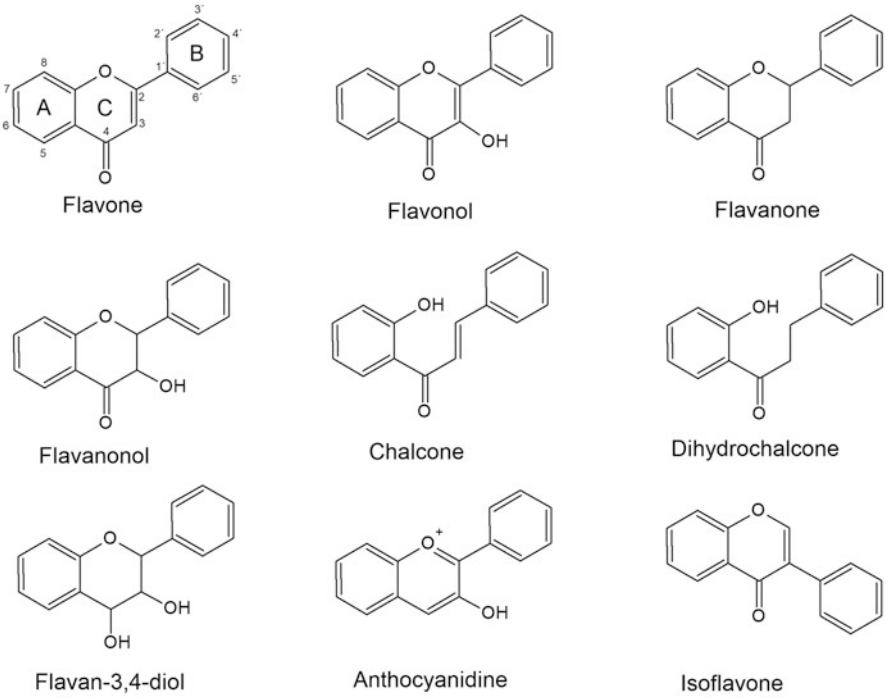


Fig. 1 Major flavonoid classes and common numbering of flavonoids

rarely in flavanols, flavonols, flavanones, dihydrochalcones, isoflavones, and isoflavanones. In C-glycosylflavones, glycosylation can occur either as mono-C-glycosylflavones (positions C-6- or C-8-), di-C-glycosylflavones (positions C-6,8-, 3,6-, 3,8-), as mixed C-/*O*-glycosides, as well as acylated glycosides, resulting in a remarkable complexity of C-glycosyl patterns. In addition, the interconversion of 6-C- and 8-C-glycosylflavones via Wessely-Moser rearrangement which can already occur during boiling has to be considered (Courts and Williamson 2015).

5.2.1.1 Analysis of C-Glycosylflavonoids

Analysis of CGF in food sources is above all accomplished by HPLC coupled to photodiode array detection and mass spectrometry usually applying electrospray ionization (ESI). The C-glycosidic linkage obviously has higher stability in mass spectrometric fragmentation processes leading in the first step not to the aglycone as in case of *O*-glycosylflavonoids by cleavage of the hemi-acetal C–O bonds, but to fragmentation of the sugar moiety when medium fragmentation energy is applied.

Intraglycosidic cleavages (Fig. 2) and losses of water can be observed. For instance, as outlined by Vukics and Guttman (2009), in product ion spectra of protonated vitexin mainly losses of 1–3 water molecules as well as $^{0,2}X^{+}$, $^{0,2}X^{+}-H_2O$, $^{0,4}X^{+}-2\ H_2O$, and $^{2,3}X^{+}-2\ H_2O$ fragments could be found, whereas in negative ion mode $^{0,2}X^{-}$ was the major fragment.

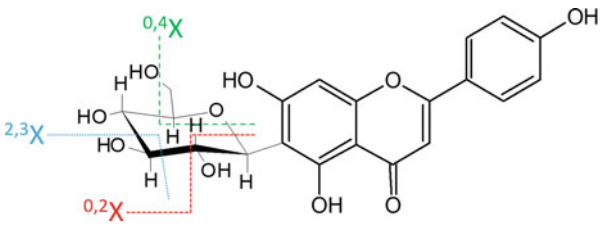


Fig. 2 Most frequently occurring intraglycosidic cleavages observed in mass spectral analysis of C-glycosyl flavones. ^{a,b}X: a, b indicate the broken sugar bonds; X indicates fragments where the charge is kept on the aglycone

Significant simultaneous losses of −90 and −120 mass units in case of hexoses and −60 and −90 u in case of pentoses can be used by applying neutral loss scans to detect CGF in complex food matrix. Also, in case of di-C-glycosyl flavones with different monosaccharides attached to the aglycone LC-ESI-MS with stepwise fragmentation will help in identification. Further, it has been shown that due to hydrogen bonding in case of a 6,8-di-C-glycosylflavone, the loss of a fragment −60 u if the pentose is attached at position C-6 is higher compared to a pentose attached to position C-8. However, it has to be considered that relative abundances of fragments depend on type of instrumentation and sound conclusions can only be drawn when spectra of C-6- and C-8-pentosylglycosides can be compared under identical instrumental settings (Waridel et al. 2001; Ferreres et al. 2003; Vukics and Guttman 2009).

5.2.1.2 Biosynthesis of C-Glycosylflavones

The biosynthetic pathway of C-glucosylflavones in cereals has been studied in detail by Brazier-Hicks et al. (2009). The flavanone precursor is converted to a 2-hydroxyflavanone and its corresponding open-chain form. A C-glucosyltransferase (CGT) forms 2-hydroxyflavanone C-glucosides by attaching a glucose moiety either at position C-6 or C-8. Through dehydration the flavone-6-C-glucoside and flavone-8-C-glucoside is finally formed, respectively. Most of the CGF identified so far in plants are members of the UGT708 subfamily which are able to C-glycosylate 2,4,6-trihydroxyacetophenone-like structures (e.g., 2-hydroxyflavanones). In a number of food plants, such CGF were found, such as rize, maize, buckwheat, citrus fruits, kudzu, and soy bean. However, also direct formation of 6-C-glycosylflavones or isoflavones by CGTs belonging to different UGT (UDP glycosyltransferase) subfamilies, i.e., UGT84 and UGT71, has been described for gentian (*Gentiana triflora*), kudzu and wasabi (*Eutrema japonicum*) (Mashima et al. 2019).

5.2.2 Food Sources of C-Glycosyl Flavonoids

A condensed summary of C-glycosylflavonoids detected in plant food sources can be depicted from Table 1. The most common structures of CGF in food are presented in Table 2 and Fig. 3.

Table 1 Food sources of C-glycosylflavonoids

Food source	Plant	Flavonoid class	C-Glycosylflavonoids	References
Fruits and seeds				
Cereals				
Wheat	*Triticum sps.*	C-Glycosylflavones	Apigenin-6-C-, 8-C-, 6,8-di-C-glycosides (including acylated derivatives)	(Courts and Williamson 2015; Wijaya and Mares 2012)
Barley	*Hordeum vulgare*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides	(Courts and Williamson 2015; Xiao et al. 2016)
Oat	*Avena sativa*	C-Glycosylflavones	Luteolin-6-C-, and 6,8-di-C-glycosides	(Popovici and Weissenboeck 1976)
Rye	*Secale cereale*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides	(Courts and Williamson 2015)
Spelt	*Triticum aestivum* subsp. *spelta*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides	(Hostetler et al. 2017)
Buckwheat	*Fagopyrum esculentum*	C-Glycosylflavones	Apigenin- and luteolin-6- and 8-C-glycosides	(Brazier-Hicks et al. 2009; Courts and Williamson 2015; Hostetler et al. 2017)
Rice plant	*Oryza sativa*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides, chrysoeriol-6-C-glycosides (including acylated derivatives), luteolin-6,8-di-C-glycosides	(Courts and Williamson 2015; Xiao et al. 2016; Hostetler et al. 2017)
Rice fruit	*Oryza sativa*	C-Glycosylflavones	Apigenin- and luteolin-6,8-di-C-glycosides	(Pereira-Caro et al. 2013; Hostetler et al. 2017)
Maize	*Zea mays*	C-Glycosylflavones	Maysin; maysin analogues; derhamnosyl-maysin	(Elliger et al. 1980; Xiao et al. 2016)
Millet	*Sorghum bicolor*	C-Glycosylflavones	Apigenin-6-C-and 8-C-glycosides; luteolin-8-C-glycosides	(Courts and Williamson 2015)
Pearl millet	*Pennisetum glaucum (syn. Pennisetum americanum)*	C-Glycosylflavones	Apigenin- and luteolin-8-C-glycosides	(Boncompagni et al. 2018; Courts and Williamson 2015)

Citrus fruits				
Bergamot	*Citrus bergamia*	C-Glycosylflavones	Apigenin-6,8-di-C-glucoside, diosmetin-6,8-di-C-glucoside	(Caristi et al. 2005; Abad-Garcia et al. 2012)
Grapefruits	*Citrus paradisi*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides	(Abad-Garcia et al. 2012; Wang et al. 2017)
Kumquats	*Fortunella japonica*	C-Glycosylflavones, C-glycosyl-dihydrochalcones	Acacetin-6-C- and 8-C-glycosides, apigenin-8-C-neohesperidoside, phloretin-3′,5′-di-C-glucoside	(Lou et al. 2016; Xiao et al. 2016)
Lemons	*Citrus limon*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides, chrysoeriol-6,8-di-C-glycosides, diosmetin-6-C-, 8-C- and 6,8-di-C-glycosides	(Abad-Garcia et al. 2012; Courts and Williamson 2015; Wang et al. 2017)
Limes	*Citrus aurantiifolia*	C-Glycosylflavones	Diosmetin-6-C- and 6,8-di-C-glucoside	(Caristi et al. 2005; Courts and Williamson 2015)
Mandarins, tangerines	*Citrus reticulata*	C-Glycosylflavones	Apigenin-6,8-di-C-glucoside, diosmetin-6,8-di-C-glucoside	(Hostetler et al. 2017)
Oranges (sweet oranges, blood oranges)	*Citrus sinensis*	C-Glycosylflavones	Apigenin-6-C-,8-C- and 6,8-di-C-glycosides, chrysoeriol-8-C-glucoside, chrysoeriol-6,8-di-C-glucoside (stellarin-2), diosmetin-6,8-di-C-glucoside	(Caristi et al. 2005; Abad-Garcia et al. 2012; Courts and Williamson 2015; Barreca et al. 2016; Hostetler et al. 2017; Wang et al. 2017)
Oranges (sour oranges)	*Citrus aurantium*	C-Glycosylflavones	Apigenin- and luteolin-6,8-di-C-glucoside (lucenin-2, vicenin-2), diosmetin-6,8-di-C-glucoside	(Barreca et al. 2010)
Pummelo	*Citrus grandis*	C-Glycosylflavones	Apigenin 6-C- and 6,8-di-C-glycosides (including acylated derivatives), diosmetin-6.C- and 6,8-di-C-glycosides, luteolin-6,8-di-C-glucoside	(Abad-Garcia et al. 2012; Zhang et al. 2014; Wang et al. 2017)
Tangelo	*Citrus reticulata X C. paradisi*	C-Glycosylflavones	Apigenin- and luteolin-6,8-di-C-glucoside	(Barreca et al. 2013)

(continued)

Table 1 (continued)

Food source	Plant	Flavonoid class	C-Glycosylflavonoids	References
Legumes				
Peas	*Pisum sativum*	C-Glycosylflavones	Apigenin- and luteolin-C-glycosides	(Courts and Williamson 2015; Hostetler et al. 2017)
Fava beans	*Vicia faba*	C-Glycosylflavones	Apigenin-C-glycosides	(Hostetler et al. 2017)
Chick peas	*Cicer arientinum*	C-Glycosylflavones	Luteolin C-glycosides	(Hostetler et al. 2017)
Mung beans	*Vigna radiata*	C-Glycosylflavones	Apigenin-6-C- and 8-C-glycosides	(Xiao et al. 2016)
Other fruits and seeds				
Passion fruits (pericarp)	*Passiflora sps.*	C-Glycosylflavones	Apigenin-6-C-, 8-C- and 6,8-di-C-glycosides, luteolin-6-C-, 8-C-glycosides, chrysin-6,8-di-C-glucoside	(Zucolotto et al. 2012)
Cayenne pepper	*Capsicum annuum*	C-Glycosylflavones	Apigenin-6-C-glycoside, luteolin-6-C- and 6,8-di-C-glycosides	(Materska 2015)
Dates	*Phoenix dactylifera*	C-Glycosylflavones	Apigenin-di-C-hexoside	(Xiao et al. 2016)
Tomatoes	*Solanum lycopersicum*	C-Glycosyl-dihydrochalcones	Phloretin-3′,5′-di-C-glucoside	(Courts and Williamson 2015)
Chayote	*Sechium edule*	C-Glycosylflavones	Apigenin-6-C- and 6,8-di-C-glycosides	(Siciliano et al. 2004)
Cucumber	*Cucumis sativus*	C-Glycosylflavones	Apigenin- and luteolin-6-C- and 8-C-glycosides, cucumerin A, B (phytoalexins)	(McNally et al. 2003)
Carob seed germ flour	*Ceratonia siliqua*	C-Glycosylflavones	Apigenin 6-C-, 8-C- and 6,8-di-C-glycosides (including acylated derivatives)	(Picariello et al. 2017)
Carambola fruit	*Averrhoa carambola*	C-Glycosyl-dihydrochalcones; C-glycosylflavones	Apigenin-6-C-glycosides (carambolaflavones)	(Wang et al. 2018)
Fenugreek (seed germs)	*Trigonella foenum-graecum*	C-Glycosylflavones	Apigenin- and luteolin-6-C-, 8-C- and 6,8-di-C-glycosides	(Xiao et al. 2016)

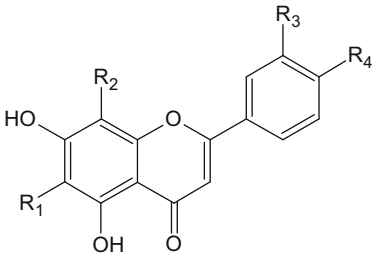
Mesquite (South American algarrobo)	*Prosopis spp.*	C-Glycosylflavones	Apigenin-6,8-di-C-glycosides (including feruloylate derivatives)	(Picariello et al. 2017)
Quince (seeds)	*Cydonia oblonga*	C-Glycosylflavones	Apigenin-, chrysoeriol- and luteolin-6-C-, 8-C- and 6,8-di-C-glycosides	(Ferreres et al. 2003)
Cocoa (processed)	*Theobroma cacao*	C-Glycosylflavan-3-ols	Catechin- and epicatechin-C-glycosides	(Stark and Hofmann 2006; Courts and Williamson 2015)
Figs (skin)	*Ficus carica*	C-Glycosylflavones	Luteolin-6,8-di-C-glycoside	(Vallejo et al. 2012)
Leaves and stems				
Bamboo shoots (leaves)	*Different species*	C-Glycosylflavones	Apigenin-C-glycosides, luteolin-8-C-glycosides	(Zhang et al. 2007)
Beet root and leaves	*Beta vulgaris*	C-Glycosylflavones	Apigenin-8-C-glycosides	(Courts and Williamson 2015; Ninfali et al. 2017)
Honeybush herbal tea	*Cyclopia sps.*	C-Glycosylflavones, C-glycosyldiydrochalcones	Phloretin-3′,5′-di-C-glucoside, vicenin-2, 3-hydroxyphloretin-3′,5′-di-C-hexoside	(Schulze et al. 2015)
Lemongrass leaves	*Cymbopogon citratus*	C-Glycosylflavones	Apigenin-6-C-, 8-C- and 6.8-di-C-glycosides, luteolin-6-C-, 8-C- and 6.8-di-C-glycosides	(Figueirinha et al. 2008)
Lotus (different plant parts)	*Nelumbo nucifera*	C-Glycosylflavones	Apigenin- and luteolin-6-C- and 8-C-glycosides; especially rich in Lotus plumules (all other parts contain less CGFs)	(Li et al. 2014)
Palm products (palm leaves etc.)	*Different species*	C-Glycosylflavones	Apigenin-6- and 8-C-glycosides, luteolin-6-C- and 8-C-glycosides	(Williams et al. 1973)
Pigeon pea	*Cajanus cajan*	C-Glycosylflavones	Apigenin-8-C-glycosides, luteolin-8-C-glycosides	(Nix et al. 2015)

(continued)

Table 1 (continued)

Food source	Plant	Flavonoid class	C-Glycosylflavonoids	References
Rooibos herbal tea	*Aspalathus sp.*	C-Glycosyl-dihydrochalcones, C-glycosylflavanones, C-glycosylflavones	Aspalathin, nothofagin, eriodictyol-C-glycosides, apigenin- and luteolin-6-C- and 8-C-glucosides	(Courts and Williamson 2015; Hostetler et al. 2017)
Sugar cane	*Saccharum officinarum*	C-Glycosylflavones	Apigenin-6-C-, 8-C- and 6,8-di-C-glycosides, luteolin-8-C-glycosides, 4′,5′-dimethyl-luteolin-8-C-glycoside	(Courts and Williamson 2015)
Taro (leaves)	*Colocasia esculenta*	C-Glycosylflavones	Apigenin-, chrysoeriol-, diosmetin-, luteolin-6-C-, 8-C-, 6,8-di-C-glycosides	(Ferreres et al. 2012)
Tea (green, oolong, white, black)	*Camellia sinensis*	C-Glycosylflavones	Apigenin C-glycosides, luteolin C-glycosides	(Hostetler et al. 2017)
Rhubarb	*Rheum sp.*	C-Glycosylflavones	Apigenin-6-C- and 6,8-diC-glycosides	(Courts and Williamson 2015)
Roots				
Beet root and leaves	*Beta vulgaris*	C-Glycosylflavones	Apigenin-8-C-glycosides	(Courts and Williamson 2015; Ninfali et al. 2017)
Kudzu	*Pueraria montana*	C-Glycosylisoflavones	Daidzein 8-C-glucoside (puerarin), 3′-OH-puerarin, 3′-OMe-puerarin, 5-OH-puerarin	(Wu et al. 2011)

Table 2 Structures of most common C-glycosylflavones and aglycones



Compound	R_1 (C-6)	R_2 (C-8)	R_3 (C-3′)	R_4 (C-4′)
Apigenin	H	H	H	OH
Luteolin	H	H	OH	OH
Chrysoeriol	H	H	OCH_3	OH
Diosmetin	H	H	OH	OCH_3
Vitexin	H	β-D-gluc	H	OH
Isovitexin	β-D-gluc	H	H	OH
Orientin	H	β-D-gluc	OH	OH
Isoorientin	β-D-gluc	H	OH	OH
Vicenin-2	β-D-gluc	β-D-gluc	H	OH
Lucenin-2	β-D-gluc	β-D-gluc	OH	OH
Schaftoside	α-L-ara(p)	β-D-gluc	H	OH
Isoschaftoside	β-D-gluc	α-L-ara(p)	H	OH

β-D-gluc = C-β-D-glucosyl-; α-L-ara(p) = C-α-L-arabinopyranosyl-

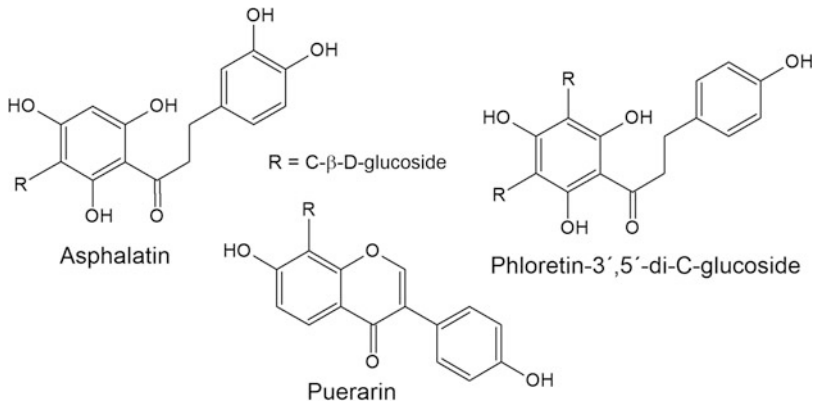


Fig. 3 C-Glycosylflavonoids other than flavones: asphalatin and phloretin-3′,5′-di-C-glucoside as examples of C-glycosyldihydrochalcones, puerarin representing a C-glycosylisoflavone

As shown in Table 1, by for the majority of CGF in food plants are present as C-glycosylflavones, followed by C-glycosyldihydrochalcones, main food resources of this type of compounds are tomatoes (phloretin-3′, 5′-di-C-glucoside) and rooibos tea (aspalathin, nothofagin). Fermentation (heating and/or sundrying) of rooibos tea forms C-glycosylflavanones (eriodictyol glycosides), and further oxidation leads to

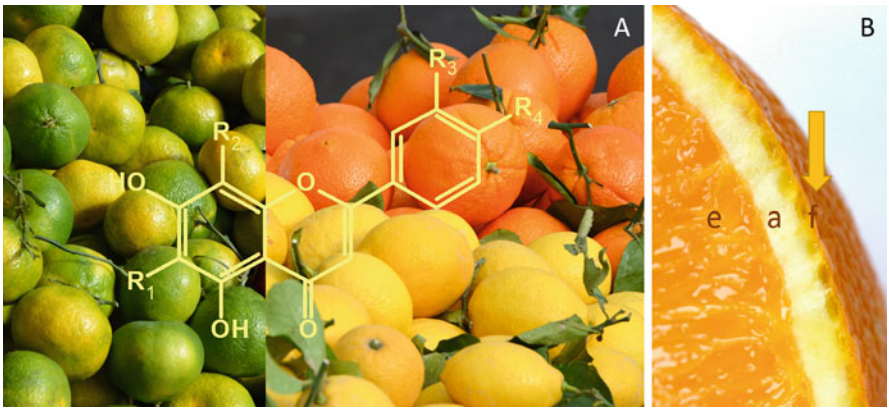


Fig. 4 (**A**) Citrus fruits like oranges, lemons, and mandarins are a major food source of C-glycosyl flavones. (**B**) C-Glycosylflavones can mainly be found in the outer flavedo layer (f) whereas lower amounts are present in the albedo layer (a) and endocarp (e)

C-glycosylflavones. Similar as in rooibos tea, C-glycosylflavan-3-ols in cocoa seeds are resulting from the fermentation process (Courts and Williamson 2015).

In order to evaluate the relevance of CGF as compounds in food and their possible impact on human health, of course their amounts in the diet have to be known. However, data concerning quantities, especially in processed food, are scarce. The following data refer to reported quantities summarized by Hostetler et al. (2017). Citrus fruits represent a major source of C-glycosylflavones (Fig. 4), with total quantities of 1.6–9.2 mg/100 g fresh weight (corresponding to 6–23 mg per serving) in orange juice, 8.6–15.4 mg/100 g fresh weight in fruit juices of bergamot, 2.9–3.5 mg/100 g in mandarin juice, and 6.7–7.6 mg/100 g fresh weight in lemon juice. Investigations of commercials juices of pink grapefruit (*C. paradisi*) and oranges (*C. sinensis*) showed quantities of vicenin-2 (apigenin-6,8-di-C-glucoside) of 29.3–45.3 mg/L (Bucar, unpublished data). Investigations of accumulation patterns of C-glycosylflavones in various tissues and *Citrus* species revealed the highest proportions in the flavedo layer (colored outer layer of the pericarp) and significantly less in the albedo layer (inner whiteish layer of the pericarp), segment membranes, and fruit juice (Wang et al. 2017), see also Fig. 4.

In a wheat grain-based meal, the estimated amount of C-glycosylflavones is relatively low ranging between <1–5 mg per serving (28 g dry grain). Our own exploratory analysis of apigenin-6,8-di-C-glycosides in spelt (*Triticum aestivum* subsp. *spelta*) pointed towards preferable intake of wholemeal flour (66 mg/kg) compared to plain flour (32 mg/kg), and amounts in wheat (*Triticum aestivum*) semolina were comparably low (6 mg/kg) (Bucar, unpublished results). According to Hostetler et al. (2017), this is also true for different types of rice (amounts of apigenin and luteolin-C-glycosides below <1 mg per serving (28 g) and dehulled, roasted, as well as whole buckwheat (<1 mg per serving). Similar low amounts are reported for legumes like field peas (*Pisum sativum*), i.e., <1–1 mg per 28 g serving, and chick peas (<1 mg per serving). However, unexpectedly high amounts of vitexin (apigenin-8-C-glucoside) equivalents were detected in flour of millet seeds with

77–275 mg/100 g (Courts and Williamson 2015), however determined by a spectrophotometric method without further confirmation after chromatographic separation. A study of Boncompagni et al. (2018) on grains of 96 pearl millet lines from a panel of inbred lines covering a large genetic diversity revealed also highly divers quantities of C-glycosylflavones. Ranges of 0 to 283.4, 272.7, and 261.1 mg/kg (mean values of 71.9, 28.1, and 40.0) were found for glucosylvitexin, orientin, and vitexin, respectively. Extremely high amounts of different apigenin-C-glycosides were reported for carob seed germ flour summing up to more than 8.3 g/kg in total (Picariello et al. 2017).

Although high quantities of apigenin- and luteolin-C-glycosides (up to 246.6 mg/100 g dry weight) have been found in different processed teas (green, black, oolong teas of *Camelia sinensis*), in the final infusion only <1–4 mg per serving (corresponding to 2 g dry material) can be expected. Flavonoids and phenolics in tea are covered in more detail in ▶ Chaps. 18, "Tea Catechins" (Daglia and Baldi), and ▶ 40, "Procyanidins in Food" (Sieniawska et al.).

Tomatoes and infusions of rooibos herbal tea (*Aspalathus linearis*) are the main food source of C-glycosyldihydrochalcones (Courts and Williamson 2015). In investigated tomato cultivars, phloretin-3′,5′-di-C-glucoside contributed by 5–14% to the total flavonoid content which varied from 4 to 26 mg per 100 g fresh weight. Further relevant sources of this C-glycosyldihydrochalcone are the fruit peels of mature kumquats (*Fortunella japonica*) showing highly remarkable 1.35 g per 100 g dry weight (Lou et al. 2016), as well as honeybush herbal tea (*Cyclopia spp.*) with 0.77–13.3 mg/L hot water infusion (Schulze et al. 2015).

In rooibos (leaves and stems), a significant difference in unprocessed and fermented herbs can be observed. Whereas unfermented freeze-dried rooibos tea contains about 1.5 g aspalathin and 0.43 g nothofagin per 100 g, due to oxidative ring cyclisation to eriodictyol-C-glycosides, the amount of both dihydrochalcones declines to 102 mg and 35 mg per 100 g, respectively, in fermented rooibos tea. In addition, the C-glycosylflavones luteolin-6-C-glucoside (isoorientin) and luteolin-8-C-glucoside (orientin) have been extracted to aqueous infusions of fermented rooibos tea at quantities of 100 and 80 mg per 100 g, whereas the corresponding apigenin glucosides were only present in lower amounts of 33 and 27 mg per 100 g, respectively (Courts and Williamson 2015; Hostetler et al. 2017).

As most prominent source of C-glycosylisoflavonoids (mainly puerarin), kudzu (Japanese arrowroot, *Pueraria montana var. lobata*) has to be mentioned. According to Wu et al. (2011), its dried roots contained 1.97% of puerarin, whereas other puerarin derivatives were present only in low quantities (0.024–0.16%). Structures of most common flavone C-glycosides are shown in Table 2, and C-glycosylflavonoids other than flavones are presented in Fig. 3.

5.3 Bioavailability

CGF have a higher stability compared to their *O*-glycosylflavonoids (OGF) in regard to their hydrolysis rate. Human studies reveal that flavonoid C-monoglycosides (such as vitexin, isovitexin, orientin, isoorientin, and puerarin, for structures see

Table 2 and Fig. 3) are only absorbed to a minor extent. Metabolism of CGF differs mainly from that of *O*-glycosylated flavonoids which are hydrolyzed easier by acid and enzymes compared to CGF. Therefore, CGF are not affected to the extent as seen for OGF by hepatic and gastrointestinal hydrolysis obvious due to the appearance of intact CGF in urine. No mammalian enzymes which are capable of cleaving the C-glycosidic linkage are known; therefore, resident bacteria in the human colon are the only metabolism step experienced by CGF. With a bamboo extract (containing vitexin, isovitexin, orientin, isoorientin) administered to rats, it was found that these CGF are poorly absorbed from the gastrointestinal tract and are therefore passed to colon which were then excreted by feces within 24 h. After examination of rats after 12 h of administration, no CGF were found in liver and brain. Microbial degradation gave rise to metabolites like phloroglucinol, hydrocaffeic, and phloretic acid. In contrast, C-multiglycosides of flavonoids are absorbed from the small intestine in unmodified nature and distributed to the liver. From there, CGF are either infiltrated into the system circulation and distributed to various tissues or directed to urinary excretion (Xiao et al. 2016). Hence, deglycosylation does not seem to be essential for CGF absorption (Courts and Williamson 2015).

Bioactivity of such phenolic compounds is strongly dependent on the release rate from the matrix (bioaccessibility), alterations during the passage of gastrointestinal tract, and the principal metabolism in vivo. Bioaccessibilty of dietary flavonoids is again dependent on many factors such as matrix, filling of stomach, liquid consumption during eating, pH value of gastrointestinal tract, peristalsis, and blood and lymph flow. On the other hand, it is dependent on the nature of the flavonoid – nature and scale of glycosidation, type of sugar, and how it is linked to the aglycone. The interaction of flavonoids and proteins is feasible after what they are less likely to be ready for digestion. The main reactions going on in the liver include methylation, sulfatation, and glucuronidation (Czubinski et al. 2019).

Glycosides can be seen as prodrugs enhancing the solubility of their aglycones. Only a small extent of flavonoid glycosides is absorbed in the small intestine – the majority is transported to the large intestine and there deglycosylated by colonic bacteria takes place. Once they are absorbed, they are metabolized by phase II enzymes and then conveyed into the liver. Hydroxylation, reduction, or methylation take place to release the aglycones which in turn get further metabolized (sulfated/glucuronidated) to the typical flavonoid metabolites (with improved solubility and molecular weight) which circulate in the body.

Especially the enzymes of UDP-glucuronosyltransferases 1 and 2 family are responsible for the glucuronidation of flavonoids. Local release of aglycones from glucuronidated species has been observed in inflammatory cells, such as macrophages which release lysosomal enzymes, including β-glucuronidase, which can lead to deconjugation in the tissues and uptake of aglycones by the cells to a much higher rate compared to the glucuronide conjugates. The in situ deconjugation has been pointed as an absolute requirement to the flavonoid bioactivity by delivering the free aglycone to the tissues, which is the final effector (Xiao et al. 2016; Xiao 2017) (Fig. 4).

More than 400 bacterial species can habitat a human body and therefore the metabolization rate differs from one person to another. Little is known for the

deglycosidation processes for CGF although some bacterial strains were reported to be involved in the cleavage of C-glycosides; among them are strains from *Enterococcaceae*, *Lachnospiraceae*, and *Streptococcaceae*. The specific strain responsible for the cleavage of C-coupled sugar is noted in the sections below assigned to the affected compound. It is further reported that most bacteria able to cleave the C-linkage are also able to act on *O*-glycosides. Bacteria could be responsible for one step in the metabolization or can perform the whole cascade to degradation products (Braune and Blaut 2016). Degradation of isoorientin by human intestinal bacteria was studied by Hattori et al. (1998).

The binding of ingested compounds to serum albumin is an important factor how pursuing distribution, excretion, and metabolism is carried out in human body. Serum albumin is known for its function as carrier and storage place for various compounds of endogenous and exogenous nature (such as fatty and amino acids, steroid hormones, and drugs) and therefore is of critical importance for bioavailability and duration of drug action. A study conducted by Cao et al. examined the affinity of various flavonoids to human serum albumin by using high performance affinity chromatography. For this reason, 63 structurally different flavonoids were tested for their human serum albumin binding affinity among others 4 CGF. Apigenin-6-C-glycoside, puerarin, vitexin, and vitexin-2″-rhamnoside were tested with protein binding rates of 59.6%, 30.6%, 47.7%, and 19.1%, respectively. When drugs are bound to proteins, reversible complexes are formed from which drugs cannot diffuse through plasma membranes and no metabolization is possible. On the other hand, drugs can diffuse from these reversible complexes when drug plasma concentration is low and can therefore maintain an equilibrium. In this study, various substitution on the flavonoid basic structure was performed which allow interpretation of structural relationships. Glycosylation of flavonoids leads to a decrease of protein binding rate by 30–50%. This effect was mediated by steric hindrance and the higher polarity due to glycosylation. Compounds are bound to human serum albumin by a hydrophobic pocket which in turn is decreased by a lower lipophilicity. A high protein binding rate stands for a longer half-life and a slow elimination into plasma of these drugs (Cao et al. 2019).

5.3.1 Vitexin and Isovitexin

Vitexin was found to be deglycosylated to its aglycone apigenin by a bacterium found in human feces, *Lachnospiraceae* CG19-1 (Courts and Williamson 2015), whereas isovitexin was also deglycosylated by *Eubacterium cellulosolvens* to apigenin and 3-(4-hydroxyphenyl)propionic acid (Xiao et al. 2016) (see Fig. 5). The excretion ways of vitexin were studied with the substance vitexin-4″-*O*-glucoside by Cai et al. For this reason, mice were given 30 mg/kg vitexin-4″-*O*-glycoside either orally or intravenously and samples were collected at certain time points. In an oral dosing experiment after 24 h, original substance was recovered from urine with 17.9% and in feces with 6.3%. Intravenous administration led to a recovery rate of 4.7% (urine) and 0.8% (feces). These low recovery rates showed that vitexin-4″-*O*-glucoside have to be metabolized also by other means. The authors conclude that this glycoside has entered

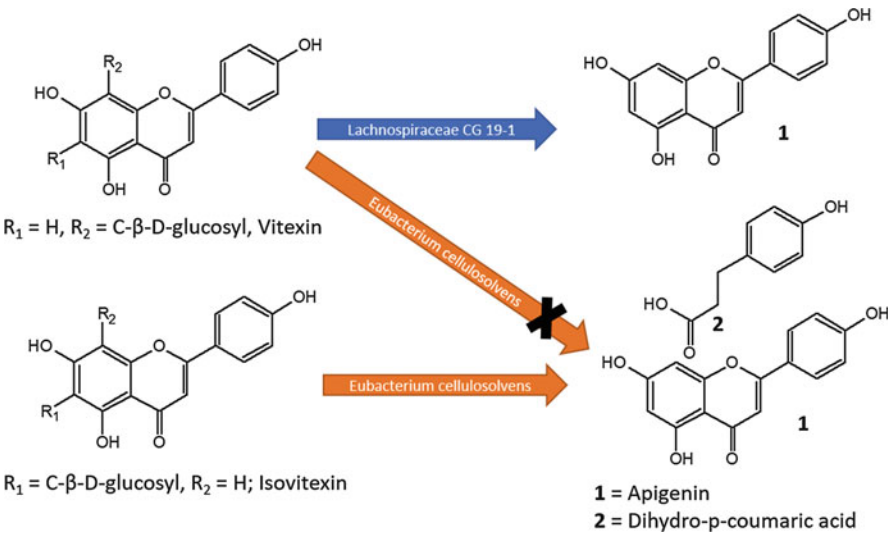


Fig. 5 Deglycosylation of vitexin and isovitexin by selected gut bacteria. Whereas Lachnospiraceae strain CG 19-1 deglycosilated vitexin to apigenin (Braune and Blaut 2016), *Eubacterium cellulosolvens* degraded only isovitexin to apigenin and dihydro-p-coumaric acid, but not vitexin (Xiao et al. 2016)

enterohepatic recirculation (elimination from the bile to the intestine and simultaneously reabsorption into the blood flow) and was then eliminated. More than four-fold higher elimination was detected after oral administration, whereas plasma concentrations were higher after i.v. administration. It can be concluded the renal way is the major excretion way of vitexin-4″-*O*-glucoside from mice (Cai et al. 2013).

A further experiment was performed with vitexin-2″-*O*-xyloside in order to gain insight into the bioavailability of CGF. The compound was administered (8.15 μmol) directly in the caecum of rats and blood samples were taken at various time points. The CGF gave rise to two peaks in the blood – on the one hand the unchanged vitexin-2″-*O*-xyloside and, on the other hand a vitexin glucuronide. This glucuronide was detectable as early as 30 s after administration. Unmetabolized drug enters enterohepatic recirculation and therefore gets reabsorbed in the intestine partly in conjugated form. The caecum was observed, showing a retarded amount of vitexin-2″-*O*-xyloside of around 25% and no conjugates were detected – therefore metabolization products by gut bacteria or enterocytes are thought to be immediately released into the blood (Xiao et al. 2016).

5.3.2 Schaftoside, Isoschaftoside, Vicenin-2

With a human gastrointestinal model, the metabolization of schaftoside and isoschaftoside was shown resulting in the information that these CGF are stable through their passage of gastrointestinal dialyze model. The same data were obtained for

vicenin-2 and vitexin. During this model, only *O*-glycosidic linkages of vitexin-*O*-glycosides were broken by colon bacteria. A search was started for specific degradation products of vitexin, but it can be considered as stable due to the fact that no metabolites (such as 3-(4-hydroxyphenyl) propionic acid) occurred over time. Data regarding active absorption and enzymatic influence is missing in this model (van Dooren et al. 2018). In an in vivo model using rats it was shown that schaftoside, isoschaftoside, and vicenin-2 were rapidly absorbed into the blood circulation. The plasma curves led to the conclusion that there are possibly two absorption sites in the intestine. The distribution of these three CGF was mainly in the kidneys followed by liver, lungs, heart, and spleen (for vicenin-2 and isoschaftoside) (Xiong et al. 2015).

Lupin seeds (*Lupinus angustifolius*) are widely used in nutrition. Isolation gave rise to the free aglycone apigenin and three CGF could be isolated from this plant whereas apigenin 6,8-di-*C*-glucoside (vicenin-2) and apigenin 7-*O*-apiosyl-6,8-di-*C*-glucoside as the two main CGF. It was found that these CGF are able to bind to proteins and during digestion can be released if these proteins are broken down. When digesting these seeds in an in vitro model, the total phenolic content decreased by 57% (at stomach level) and an additional 5% when measured at small intestine level. No qualitative change in CGF composition was found after digesting the seeds, but quantitative changes were found (a lesser amount was documented). In other *Lupinus* species a change was found in the CGF composition after digestion. In *L. luteus* no hexoside of apigenin feruloyl-7-apiosyl-6,8-di-C-glucoside was detectable and in *L. albus* no aglycone apigenin was found post digestion. In case of *L. luteus* new *O*-glycosides appear in after digestion measurements. On the example of *L. angustifolius*, it was demonstrated that 83% of apigenin 7-*O*-apiosyl-6,8-di-*C*-glucoside were released at stomach digestion and additionally 9% at intestine level. Very similar results were obtained for apigenin 6,8-di-*C*-glucoside with 86% in stomach and + 7% in the intestine. These results show that CGF are bioaccessible in a high extent. An additional experiment of protein digestion was conducted and showed that 54% of lupin protein was digested during the in vitro model. The higher the total phenolic content, the lower was the protein digestion rate. The decrease could be explained by complex formation or the inhibitory potential of phenolic compounds on proteolytic enzymes (Czubinski et al. 2019).

5.3.3 Orientin and Isoorientin

Isoorientin was found to be deglycosylated by a bacterium found in human feces, *Lachnospiraceae* CG19-1 (Courts and Williamson 2015). In a study using human intestinal bacteria, isoorientin was metabolized to phloroglucinol, luteolin, (+)-eriodictyol, 6-C-glucosyleriodictyol, and 3,4-dihydroxyphenylpropionic acid. This metabolic deglycosylation were also reported to happen together with orientin in sheep and cows mediated by *Eubacterium cellulosolvens* (Xiao et al. 2016). Orientin and isoorientin were metabolized to luteolin by *Lachnospiraceae* CG19-1 and for isoorientin a further bacterial deglycosylation way was described with *Eubacterium cellulosolvens* (Braune and Blaut 2016).

When CGF are not absorbed in the upper intestinal tract, they get degraded by intestinal bacteria to breakdown products. In this context, isoorientin is metabolized to (±)-eriodictyol-6-C-β-glucoside which in turn is reduced to luteolin and (±)-eriodictyol. The latter substance is further degraded to phloroglucin, 3-(3,4-dihydroxyphenyl)-propionic acid and 3-(3-hydroxyphenyl)-propionic acid (Webster and Wood 2016). A newly found human bacterial strain *Enterococcus* sp. 45 (affiliated with *Enterococcus casseliflavus*) was able to cleave orientin to luteolin. Furthermore, it was noted that these bacteria are able to acetylate and deoxygenate orientin. These effects were carried out by several other bacterial strains too. Furthermore, the original substance orientin could be detected (Xu et al. 2014).

5.3.4 Puerarin (Daidzein-8-C-Glucoside)

A specific bacterial *strain PUE* (affiliated with *Dorea longicatena*, *Lachnospiraceae*) was able to cleave puerarin to daidzein. This strain is regularly found in human gut microbiota. This reaction was also reported for *Lachnospiraceae* CG19-1, *Lactococcus* sp. MRG-IFC-1, and *Enterococcus* sp. MRG-IFC-1 (Braune and Blaut 2016). Furthermore, human microbiota is not only able to metabolize puerarin to daidzein and glucose but also to (3S)-equol (Xiao et al. 2016).

The CGF puerarin was found to have a 138-times lower binding rate to human serum albumin compared to the corresponding aglycone. In contrast, the binding to bovine hemoglobin was found to be 2.45 times higher compared to daidzein. And in the case of plasma proteins of type II diabetes, the CGF and the aglycone had similar affinities. When looking at the pharmacokinetics, a 7-*O*-glucoside of puerarin showed a higher plasma concentration and was longer detectable in the blood as puerarin itself (Xiao 2017).

5.3.5 Aspalathin

This is the best examined CGF so far in human and porcine studies. In these studies, methylated metabolites of aspalathin were found after oral administration. No intact aspalathin was found in the plasma of human and pig. However, in urine this substance was detected showing limitation of plasma extraction methods (eventually binding to serum albumin) and therefore, surveillance of plasma concentrations of CGF is challenging due to insensitive methods. In a bioavailability study with aspalathin, there were glucuronide and sulfate conjugates found in vivo. Furthermore, it was shown that the catechol-*O*-methyl-transferase takes action in this metabolic process by methylating the catechol moiety of aspalathin giving rise to a 3-*O*-methylaspalathin which was then detected in urinary samples. With Caco-2 cells, the passive transport by diffusion of aspalathin into the intestinal epithelium was demonstrated. During this transport, aspalathin was not deglycosylated. The uptake into enterocytes occurred only in a small manner probably by the

nonselective transport of *O*-glycosides by enterocyte glucose carrier proteins (such as SGLT-1). Aspalathin has only a poor bioavailability (Courts and Williamson 2015).

After administration of fermented and unfermented rooibos tea (*Aspalathus linearis*) to human, volunteers' urine and blood samples were collected over 24 h and evaluated. The main metabolite found after fermented tea consumption was eriodicytol-*O*-sulfate and *O*-methyl-aspalathin-*O*-glucuronide in case of unfermented tea. Further metabolites found in both teas include aspalathin-*O*-sulfate, *O*-methyl-asphalathin-sulfate, and aspalathin-*O*-glucuronides. The authors suggest the absorption of eriodicytol-*O*-sulfate in the large intestine due to the excretion via urine after 5–12 h, whereas most aspalathin metabolites were found in urine within 5 h (uptake from small intestine). No metabolites in plasma were found throughout this study (Xiao et al. 2016).

5.4 Bioactivities

In food plants, as in ethnomedically applied plants, a plethora of compounds can be responsible for the observed effects, it is not clearly possible to distinct whether a plant is solely active due to the high CGF content. Therefore, here only plants are linked to an activity which are especially rich in CGF. Betimes, only in vitro data are available.

5.4.1 Antidiabetic Activity

Due to the stable C-glycoside linkage, it is thought that this substance group is able to modulate the blood glucose level. In an in vitro experiment, model pancreatic β-cells (RIN-5F cells) were incubated with and without aspalathin (100 μM). Within 3 h, it was found that insulin secretion has risen by 30% along with no increase of cytotoxicity. The potential of this substance is thwarted by its poor bioavailability. In another study using L6 rat myotubes aspalathin was able to increase glucose uptake by 24% when used in 1 μM and by 64% in 10 μm concentration. Isovitexin was able to show in vivo an insulin secretion increase by 58% after 1 h. With this, also the glycogen values were upregulated (27%) in the muscles of these rats (Courts and Williamson 2015).

The leaves of *Microctis paniculata*, a common ingredient in Chinese herbal teas, were studied for their CGF content. Two substances, vitexin and isovitexin, were isolated from these leaves and tested for their activity to inhibit α-glucosidase. As both compounds are also frequently found in food plants, the results of this study should be presented here. The methanolic extract was able to inhibit α-glucosidase with a half maximal inhibitory concentration (IC_{50}) of 61.3 μg/ml in a concentration-dependent manner. For vitexin (see Fig. 6) and isovitexin, an IC_{50} concentration of 96.9 μg/ml (244.0 μM) and 115.1 μg/ml (266.2 μM) was obtained in the α-glucosidase assay (positive control: acarbose with 1007 μM). Therefore, the two CGF are

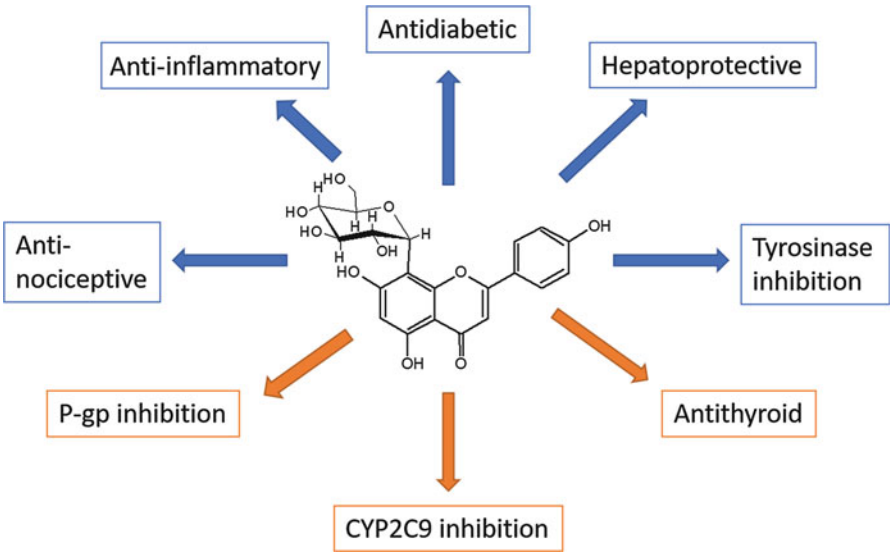


Fig. 6 Biological activities of vitexin. Activities which could cause undesired interactions with other compounds or lead to adverse effects are indicated in orange

emphasized as possible antidiabetes drug candidates (Xiao et al. 2016). The same two substances were tested in the α-glucosidase assay by another working group according to Xiao et al. (2016). They reported an in vitro IC_{50} of 4.1 μg/ml for vitexin and 6.7 μg/ml for isovitexin, which are both lower than that of acarbose. Both CGF were isolated from *Ficus deltoidea*. In addition, an in vivo experiment using normoglycemic mice and diabetic rats induced by streptozotocin was performed. Both CGF were found to lower the postprandial glucose level when measured at 30 min. Vitexin when orally administered at 1 mg/kg to normoglycemic mice significantly reduced the blood glucose level by 24.7%, and at higher concentrations of 3 mg/kg and 15 mg/kg the reduction was further enhanced to 26.5% and 31.3%, respectively. After 1 h, the glucose level returned to normal in normoglycemic mice. All three doses were equivalent to acarbose (3 mg/kg), the positive control. Extrapolation of 1 mg/kg in mice leads to an equivalent dosage of 0.08 mg/kg in humans. Isovitexin was able to lower the postprandial glucose level in normoglycemic mice at 60 min when used at 3 mg/kg and 15 mg/kg, whereas 1 mg/kg isovitexin was comparable with acarbose. In diabetic-induced rats higher concentrations of corresponding CGF were employed. The peak of postprandial glucose level in these rats was found to be at 60 min with an additional increase of 20.8%. The highest reduction (19.7%) in diabetic rats was achieved with 200 mg/kg vitexin and 100 mg/kg isovitexin, respectively. An already significant result was obtained with 50 mg/kg vitexin (human equivalent dose of 8.1 mg/kg) and 20 mg/kg isovitexin (human equivalent dose of 3.3 mg/kg). Similar effects were obtained with 5 mg/kg acarbose. Furthermore, the toxicity of oral administration of 2 g/kg vitexin and

isovitexin was measured with no signs of toxicity (mortality and weight change) after 24 h and 14 days. The dried leaves of *F. deltoidea* only contain 0.34% vitexin and 0.13% isovitexin; therefore, an infusion can only be active in patients with mild diabetes (Xiao et al. 2016). The formation of advanced glycation endproducts (AGE) is feared as a driving force in diabetic pathogenicity. It is associated with cataract, neuro-, nephro-, and retinopathy in diabetic patients. A hydroethanolic extract of mung beans (*Vigna radiata*) was found to be a potential inhibitor of this AGE product formation. 80.4% inhibition was achieved with a concentration of 500 ppm in the bovine serum albumin glucose test. In the subsequent analysis, it was found that mung beans are rich in phenolic compounds – especially vitexin and isovitexin. These two CGF were tested in the same test model at a concentration of 100 μM and were found to be slightly lower inhibitory active than rutin, an already known excellent AGE inhibitor. Researchers believe that the anti-glycation activity can be attributed to the free radical scavenging ability of these CGF (Xiao et al. 2016). Sprouts are part of a modern diet; therefore, it was documented in an experiment that fenugreek (*Trigonella foenum graecum*) and barley sprouts (*Hordeum vulgare*) could be of potential use in diabetes management. Both sprout juices were able to reduce the fasting blood glucose level compared to untreated diabetic rats. An additional effect on the lipid profile of rats was observed, whereas barley sprout juice performed best, nearly improving the lipid profile of diabetic rats to normal status (total cholesterol – 27.9%, triglycerides – 16,9 and LDL – 51.4% compared to untreated diabetic rats). Both juices were also able to reduce lipid peroxidation and oxidative stress measured by malondialdehyde levels and catalase activity (Mohamed et al. 2019). The leaves of *Centaurea alexanderina* were extracted with methanol and then screened for their constituents resulting in three reported CGF: vicenin-2, vitexin, and isovitexin, all of them can also be found in citrus fruits. With this extract and oral glucose tolerance test with normoglycemic rats was conducted. The dose of 600 mg/kg extract was able to lower the blood glucose levels by 9.4 and 10.5% when tested after 1 and 2 h, respectively. These results were just over the reference substance glibenclamide. In diabetic rats, the treatment with the extract over 30 days led to a significant decrease of blood glucose levels by 3.8% for 600 mg/kg, whereas the maximum was reached after 60 days with 4.9% (Kubacey et al. 2012).

5.4.2 Anticancer Activity

Citrus CGF were found to alter key enzymes responsible for cell activation and receptor binding and simultaneously be of low toxicity tested in animals (Xiao et al. 2016). In an experiment using isoorientin, it was documented that this CGF was able to increase apoptosis and autophagy and inhibit proliferation in human hepatoblastoma cancer cells. The induction of apoptosis was mediated by mitochondrial dysfunction, the inhibition of signals which trigger apoptosis and autophagy (such as p53 and NFκB) and also Fas-pathway was activated. Isoorientin was tested against human liver cells and no toxicity was found for this CGF using

concentrations up to 80 μM (Xiao et al. 2016). P-glycoprotein (p-gp) is a membrane protein known for its efflux capacity and therefore of importance in the resistance development of cancer cells to anticancer treatment. 75 flavonoids were screened for their ability to inhibit p-gp amongst others also three CGF: orientin, puerarin, and vitexin (see Fig. 6). In a first step it was found that all three CGF were of nontoxic nature when tested in 100 μM against MDR1-MDCKII cells. Vitexin was the best CGF to inhibit p-gp with 34.3%, followed by orientin with 20.8% and puerarin with 18.5% respectively (Bai et al. 2019). Furthermore, it was shown with esophageal cancer cells (EC-109) that orientin and vitexin were able to induce apoptosis and therefore inhibit cell growth. The oncogene expression of p53 was significantly increased after 48 h incubation in a concentration-dependent manner (up to 80 μM), whereas the effect of orientin was found to be stronger (An et al. 2015). An extract rich in vitexin and isovitexin (and chlorogenic acid) from *Trigonella foenum graecum* was tested active against human breast cancer cell line (MCF-7) and African green monkey kidney (Vero) cells with an IC_{50} of 2.5 μg/ml for each strain (Kadaikunnan et al. 2015). A mixture of CGF (vitexin, isovitexin, and orientin) was detected in a CO_2 extract of the leaves of *Cajanus cajan*. This extract was tested against three cell lines in order to evaluate the cytotoxicity: mouse macrophage cell line RAW 264.7 (68.9 μg/ml), Vero (62.5 μg/ml), and baby hamster kidney-21 cells (64.1 μg/ml). For the MCF-7 an IC_{50} value of 55.7 μg/ml was determined (Zu et al. 2010).

5.4.3 Antioxidant Activity

In general, CGF show higher antidiabetic and antioxidant activity as their equivalent O-glycosides and aglycones. CGF, mainly vitexin (see Fig. 6) and orientin, isolated from pigeon pea leaves (*Cajanus cajan*) were found to be active in DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment and was therefore successfully tested as stabilizer for blueberry juice (Xiao et al. 2016). From *Ficus deltoidea*, a traditional Malaysian tea, 16 CGF were extracted, whereas vicenin-2 (apigenin-6,8-*C*-diglucoside) was found to contribute with 9.1% antioxidative activity to the over-all effect of this tea. Other CGF do not elicit a notable antioxidant effect (Xiao et al. 2016). CGF of cayenne pepper (*Capsicum annuum*), widely used as spice, were found to act as antioxidant by superoxide radical scavenging and further were a strong inhibitor of xanthine oxidase. From these fruits, luteolin-6-*C*-glucoside, luteolin-6,8-*C*-diglucoside, and apigenin-6-*C*-glucoside-8-*C*-arabinoside were extracted. Luteolin-6-*C*-glucoside revealed the strongest antiradical-activity when tested in nonenzymatic β-nicotinamide adenine dinucleotide/phenazine methyl sulfate assay with an IC_{50} of 207.8 μM. In the xanthine oxidase test, apigenin-6-*C*-glucoside-8-*C*-arabinoside was able to elicit the strongest response with IC_{50} of 51.4 μM. In a DPPH model, only luteolin derivatives were active, whereas the apigenin derivatives were found to have no impact which can be traced back to the missing 3,4-dihydroxy group in the B-ring (Materska 2015). Barreca et al. found the juice of sour

oranges (*Citrus aurantium*) rich in CGF like lucenin-2, lucenin-2 4′-methyl ether, and vicenin-2 and was able to show a high antioxidative power mediated by reducing free radicals of DPPH by 48% and ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt) by 75%. The authors conclude that lucenin-2 is the most important CGF in consideration of total antioxidant effect due to the catechol moiety which confers stabilization to formed radicals (Barreca et al. 2010). The same working group screened various *Citrus* varieties in order to gain insight into the CGF content distribution. In this study, it was detected that the main CGF of lemon and citron (*Citrus limon* and *Citrus medica*) was lucenin-2 4'-*O*-methyl ether. In case of orange (*C. aurantium*), clementine (*C. deliciosa*), and tangerine (*C. reticulata*), vicenin-2 was found as the most abundant substance. Kumquats (*C. japonica*) showed a totally different profile with phloretin-3′,5′-di-C-glucoside as main component. The flavonoids of one juice were extracted and tested for their ability to quench free radicals. The overall flavonoid fraction was able to reduce DPPH radicals by 50% and ABTS radicals by 80%. It was found out that the fraction containing lucenin-2 and vicenin-2 was co-responsible for this excellent antioxidant effect by bearing free and highly active phenolic hydroxyl groups and the essential double bond in the pyrone C-ring (Barreca et al. 2014). The seeds of lotus (*Nelumbo nucifera*) are used in China to cook soup and are used as herbal tea. An ethanolic fraction was successfully tested as antioxidant against DPPH radicals (1476.2 μM TE (trolox equivalents)/g) and ABTS (2974.0 μM TE/g) and exerted also an anti-inflammatory activity (see below) (Chen et al. 2019). Finger millet (*Eleusine coracana*) is considered as staple in developing countries and was found to include some CGF, in particular five apigenin derivates. Variations of this grain were tested for its ability to reduce radicals of DPPH in a range of 14.2–21.5 μM TE/g and ABTS from 19.0 to 26.8 μM TE/g. No declaration of CGF to a specific antioxidant activity was drawn (Xiang et al. 2019). Another food ingredient, mung beans (*Vigna radiata*) were found to possess antioxidant activity. The best result in the DPPH radical scavenging capacity assay was found to be 16.79 μmol TE/g when extracted with acetone-water. Further antioxidant assays were performed: ferric reducing ability of plasma assay (20.43 μmol TE/g), hydroxyl radical scavenging capacity (95.59 μmol TE/g), and oxygen radical absorbance capability with 184.14 μmol TE/g. Therefore, mung beans can be considered as natural antioxidant and furthermore anti-inflammatory activities were observed by this working group. A correlation was found between antioxidant activity and the anti-inflammatory activities (Xiao et al. 2016). From the seeds and sprouts of fenugreek (*Trigonella foenum graecum*), a popular spice used worldwide two well-known CGF were isolated, namely, vitexin and isovitexin. The antioxidant power of the ethanolic extract of the seeds was found to be 41.23 μg/ml (IC_{50}) in the nitric oxide radical inhibition assay. In the β-carotene assay, 50% inhibition was achieved with an extract concentration of 22.75 μg/ml. With this assay it can be shown that the compounds found in the extract are able to reduce the by-products of lipid peroxidation. Various antioxidative assays were conducted: Further results (anticancer and antibacterial effects) were obtained for fenugreek and can be found in the respective section of this chapter (Kadaikunnan et al. 2015).

5.4.4 Hepatoprotective Activity

Isoschaftoside was found to be present in green tea leaves. In an animal testing model, 0.1% of isoschaftoside was given to rats as a dietary supplementation and no change in growth and food intake was measured. It was found that the liver injury induced by D-galactosamine was partly reversed by this CGF due to a suppression of increase of plasma alanine aminotransferase and asparatate aminotransferase (Xiao et al. 2016). The leaves of beetroots (*Beta vulgaris*) host two flavonoids which are *O*-substituted C-glycosides – namely, vitexin-7-*O*-β-D-glucopyranoside and vitexin-2″-*O*-β-D-glucopyranoside. Rat liver cells were injured with carbon tetrachloride and the hepatoprotective effect of these two CGF was measured by looking at the release rate of glutamic pyruvic transaminase. The first CGF was found to be active at 65.8% and the latter one with 56.1% when tested at a concentration of 100 μM. This effect was comparable with silibinin, a substance known for its hepatoprotective activity. It was further noticed that vitexin has an inhibitory activity on cell death in hepatocytes induced by tumor necrosis factor α (TNF-α) (Xiao et al. 2016).

5.4.5 Anti-inflammatory Activity

The abovementioned fraction of lotus (see Sect. 4.7) was further tested against RAW 264.7 cells in a concentration of 12.5–50 μg/ml and was able to significantly reduce the production of NO radicals which are part in the development of inflammation. Furthermore, the flavonoids of lotus were able to reduce the production of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, prostaglandin E2 (PGE-2), and TNF-α. Using affinity ultrafiltration LC/MS, the authors were able to identify potential cyclooxygenase (COX)-2 ligands. In the subclass of CGF vitexin was found to be the most promising COX-2 ligand (Chen et al. 2019). The working group of Kwon et al. determined the influence of glycosidation of flavonoids on nitric oxide production after lipopolysaccharide stimulation in BV2 microglial cells. It was found that most CGF were able to reduce the amount of NO produced. In relation to our topic, kaempferol-6-C-glucoside (present in lotus, figs and citrus juice) and luteolin-8-C-glucoside were still able to significantly inhibit the LPS-induced NO production. Especially the C-glycosidation in 6 and 8 position proved as superior (Xiao et al. 2016). A further aspect in inflammation is the vascular dysfunction. For this it was studied that orientin (and to a smaller extent also isoorientin) was able to inhibit the barrier disruption mediated by LPS and further blocked the leukocyte migration which leads to vascular permeability. These findings were also confirmed in an in vivo model with mice verifying the enhanced barrier integrity and reduced migration by a reduction of LPS-induced mortality. By various tests it was found out that orientin and isoorientin are able to inhibit MAPK and NFκB pathways. This study was able to draw structure-activity relationships: the glucoside position 8 (orientin) seems to be superior regarding the anti-inflammatory activity compared to the glucoside in position 6 as can be found in isoorientin. Vitexin and

isovitexin were also tested in this setting but did not elicit a positive effect due to the missing OH-group in position 3 (Xiao et al. 2016). In a study exploring the anti-inflammatory effect of the leaves of lemongrass (*Cymbopogon citratus*), a new luteolin-C,*O*-glycoside was described as cassiaoccidentalin B, and was found together with isoorientin in this plant. The glycosides were found to be less cytotoxic (up to 100 μM) compared to the aglycon luteolin. The CGF were found to possess no anti-inflammatory activity due to the decrease of activity by C-glycosylation. The effect on the activity of O-glycosides was less pronounced. It is also noted by the authors that macrophages release enzymes such as β-glucuronidase which are able to catalyze the deconjugation of flavonoids in the peripheral tissues of inflammation (Xiao et al. 2016). Various mungbean samples (*Vigna radiata*) were evaluated for their anti-inflammatory potential. For this, the production of various inflammatory markers was evaluated, namely, IL-1β, IL-6, and COX-2 in RAW 264.7 mouse macrophages. In the case of IL-1β, half of the samples of mung beans were able to inhibit the LPS-induced messenger RNA(mRNA) expression, whereas the highest inhibition ranged at 60%. A similar picture emerged when looking at the expression levels of IL-6. For COX-2, all samples were able to significantly inhibit the expression. The expression levels of IL-1β and IL-6 correlated with the anti-oxidant activity also reported from this study (see Sect. 4.7). Pure CGF vitexin and isovitexin and a physiological mixture of both as found in the beans were tested for their capacity to inhibit cytokine expression. It was shown that vitexin is of great importance for inhibiting the mRNA expression of COX-2, for both other cytokines no effect was shown and therefore other pure compounds such as phenolic acids are responsible for this phenomenon. Furthermore, no synergistic effect was achieved by the combination of both CGF (Xiao et al. 2016). A QSAR study performed with CGF and their corresponding anti-inflammatory activity showed various correlations. In general, C-glycosylation was preferable to O-glycosylation and the anti-inflammatory activity is increased by a glycosylation of ring A. In return, the activity is reduced by methoxylation of the hydroxyl groups in the flavone and the higher the polarity of C-2″-moiety is (Xiao et al. 2016), see Fig. 6.

An ethanolic extract of two varieties of muskmelon (*Cucumis melo*) was found to possess in vivo anti-inflammatory activities. By an UPLC-MS/MS setup among other flavones and flavonols, CGF such as isovitexin and isovitexin-2″-*O*-rhamnoside were found. Animals were orally pretreated with 25 and 50 mg/kg ethanolic extracts of peels and pulps of *Cucumis melo* var. *cantalupensis* and *Cucumis melo* var. *reticulatus* and inflammation was induced with carrageenan in the hind paw. All extracts were able to significantly inhibit the formation of edema after 3 h. The best result was obtained with the pulp of *Cucumis melo* var. *reticulatus* (50 mg/kg) reaching an inhibition of 69.41%. After this time point, the results of edema volume reduction were comparable with indomethacin for the pulp of *Cucumis melo* var. *reticulatus* (50 mg/kg) and the pulp of *Cucumis melo* var. *cantalupensis* (25 and 50 mg/kg). The extracts were also found be able to reduce pro-inflammatory cytokines like IL-1β, IL-6, PGE-2, and TNF-α in various extents (Ezzat et al. 2019). Another study conducted by Borghiet al. focused on the effect of the pure compound vitexin. Various assays were performed to evaluate the anti-

inflammatory and antinociceptive (for further details see section below) mechanisms of vitexin. In the carrageenan assay, this CGF was able to reduce the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-33, and TNF-α) and in the same time to induce IL-10 which is known as anti-inflammatory marker. Therefore, it can be concluded that vitexin is able to reduce inflammatory pain (Borghi et al. 2013). An extract rich in vicenin-2, schaftoside and isoschaftoside was administered to rats in a carrageenan paw swelling model in a concentration of 25–100 mg/kg. This treatment with the extract significantly reduced the nonspecific inflammation, whereas the highest swelling was maintained after 4 h. In the highest concentration (100 mg/kg), the extract was superior compared to the well-known anti-inflammatory drug aspirin, which was used as control substance (Xiong et al. 2015).

5.4.6 Antimicrobial/Antiprotozoal Activity

From the seeds and sprouts of fenugreek (*Trigonella foenum graecum*), a popular spice used worldwide, two well-known CGF were isolated, namely, vitexin and isovitexin. Gram-positive bacteria were susceptible for this extract and showed good activity whereas gram-negative bacteria were of low activity. Highest inhibition zones were achieved by the highest concentration tested (1000 μg of extract). Therefore, especially *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were susceptible to the fenugreek extract. In a smaller extent, the extract was also active against *Salmonella typhi* and *Escherichia coli*. Furthermore, various fungi were tested and it appeared that *Candida albicans*, *Penicillium notatum*, and *Aspergillus flavus* were vulnerable by these extracts (Kadaikunnan et al. 2015). Various flavonoids were tested against *Trypanosoma* and *Leishmania* strains. Vitexin was only moderately active against *T. brucei rhodesiense* with an IC_{50} of 55.7 μg/ml, whereas the aglycone apigenin was active against all three tested strains with lower IC_{50} values (Xiao 2017). In the leaves of the Brazilian plant *Serjania erecta*, two CGF were identified – vitexin and isovitexin. An ethanolic extract was produced and tested against various bacterial strains. For subsequent bacterial strains (minimal inhibitory concentration (MIC)) values of $\leq$15 μg/ml were detected: *Pseudomonas aeruginosa* (performed the best with MIC of 5 μg/ml), *Escherichia coli*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus*. No activity was found for *Mycobacterium tuberculosis* (MIC of 128 μg/ml) (Cardoso et al. 2013). The leaves of *Centaurea alexanderina* were extracted with methanol and then screened for their constituents resulting in three reported CGF: vicenin-2, vitexin, and isovitexin. Various extracts of this plant were screened against a variety of bacterial strains, whereas only *Pseudomonas aeruginosa* was susceptible against the methanolic extract with an inhibition zone of 40 mm (Kubacey et al. 2012). An extract from *Carissa opaca,* which was found to be rich in vitexin, isovitexin, and orientin, was tested against various bacterial strains. *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* were susceptible to a varying extent with a MIC of $\leq$5 mg/ml. No breakdown of components to a distinct activity was done in this study.

Furthermore, the inhibition of various fungi, i.e., *Aspergillus flavus*, *A. fumigatus*, and *A. niger* was successfully tested for this extract (Sahreen et al. 2013). Another extract with the same compounds, namely, a mixture of vitexin, isovitexin, and orientin, was detected in an ethanolic and a CO_2 extract of the leaves of *Cajanus cajan*. The CO_2 extract (MIC $\leq$2.5 mg/ml) showed much lower MIC values compared to the ethanolic extract. Bacterial strains such as *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were found to be vulnerable by this plant extract. Fungi species such as *Aspergillus niger* and *Candida albicans* were also susceptible. The CO_2 extract showed a higher activity against gram-positive bacteria than gram-negative bacteria. With *S. aureus* a time-kill assay was conducted with the CO_2 extract in order to evaluate the death rate in relation to drug and time. For this, a concentration of the respective MIC value and ½ MIC was tested over 4 h. Bacterial cells were able to survive the ½ MIC concentration, but with MIC concentration (39 μg/ml) cells were dead within these 4 h. An in vivo experiment with mice was conducted where animals were infected with *S. aureus* and either administered penicillin or the CO_2 extract (30 or 60 mg/kg intragastrically). After 4 weeks the mice were sacrificed and liver and spleen weights were significantly higher as in the control group. This could be traced back to the inflammation going on in these organs. Other organs were in the same range as the control group. It is mentionable that the group which received the CO_2 extract had reduced inflammation rates, even at the low dose. Therefore, it can be concluded that this extract works in vivo and no direct damage is originating from the extract (Zu et al. 2010).

5.4.7 Antinociceptive Activity

Urtica circularis, a herb cultivated in Argentina, is added to food due to its high content of minerals and vitamins. An ethanolic extract was produced from the aerial parts and a HPLC measurement showed the presence of mainly vicenin-2 but also a small amount of vitexin in the extract. In an experiment where pain was induced by formalin and the licking time of mice was observed, the extract was administered intraperitoneally at 30 mg/kg and 100 mg/kg doses and an additional 500 mg/kg by oral route. The licking time, checked between 15 and 30 min after exposure, was markedly reduced by 78.5%, 91.0% and 88.5% respectively. For this, an ED_{50} of 15.8 mg/kg was calculated. When active in the late phase, substances are attributed to act as antinociceptives by peripheral mechanisms (no involvement of the opioid system). By testing the extract in combination with atropine, an involvement of the cholinergic system could be documented. The writhing test, where pain was induced by acetic acid, was also performed and prior testing the mice were administered i.p. with 10–300 mg/kg *Urtica* extract. The extract dose-dependently inhibited the writhing of mice, whereas the maximum of 80.6% inhibition was achieved with the 300 mg/kg dose surpassing the effect of 10 mg/kg indomethacin i.p. (ED_{50}: 72.2 mg/kg). The 500 mg/kg p.o. dose inhibited the writhing by 48.4%. The experiments showed that the combination of *Urtica* extracts in not effective

concentrations (50 and 100 mg/kg p.o.) with indomethacin (3 mg/kg p.o., also no effect) gave rise to a promising antinociceptive effect. Furthermore, pure compounds found in the extract were tested as control. 10 mg/kg i.p. of vitexin and vicenin-2 were administered and resulted in an inhibition of 91% (again superior compared to indomethacin 10 mg/kg) and 41%, respectively. In contrast, apigenin did not elicit any effect. As gastrointestinal side effects often occur with antinociceptive drugs, the effect of the extract was observed on the stomach were only mice administered with 300 mg/kg and indomethacin 10 mg/kg were tested positive for lesions in the stomach (extract: 71% vs. indomethacin: 100%). When administered with 100 mg/kg *Urtica* extract, no difference was observed to control group which were fed only with water. Furthermore, the acute toxicity of the *Urtica circularis* extract was tested in oral concentrations up to 3000 mg/kg and no signs of toxicity were observed (Gorzalczany et al. 2011). This effect of vitexin was confirmed by another working group. Mice were pretreated with 0.3–10 mg/kg of vitexin i.p. and 30 min afterwards writhing was induced by acetic acid which led to a dose-dependent inhibition of writhing score. Another assay with phenyl-p-benzoquinone was performed where also the writhing was inhibited by vitexin. For this reason, a participation of nociceptive mechanisms which are attributed in both assays such as prostanoids, IL-33, and endothelin-1, is feasible (Borghi et al. 2013). The leaves of *Centaurea alexanderina* were extracted with methanol and then screened for their constituents resulting in three reported CGF: vicenin-2, vitexin, and isovitexin. Writhing was induced with acetic acid and mice were pretreated with doses of 300 and 600 mg/kg leaves extract. These doses significantly reduced the writhing by 35% and 49%, respectively, whereas aspirin (100 mg/kg) as reference compound reached 72%. An additional experiment where a nociceptive irritation was induced by formalin was conducted. Especially the second pain phase involving inflammation was significantly reduced by 47% (300 mg/kg) and 58% (600 mg/kg), respectively. Aspirin again inhibited paw licking by 71%. In the tail flick test, only the higher concentration of 600 mg/kg extract was able to inhibit the mechanism significantly. In the hot plate test, the extract was able in both concentrations to delay the thermal irritation but was less pronounced as with tramadol as positive control substance. One last test was performed inducing edema with carrageenan. 600 mg/kg of extract was able to protect 41% of the edema production after 4 h and was therefore significant. As positive control indomethacin was used in this experiment showing a protection of 48%. An inhibition of the prostaglandin production is discussed as the analgesic effect of this plant. With all these five tests, it can be concluded that *C. alexanderina* is able to peripherally (writhing and paw licking) and centrally (tail flick and hot plate) inhibit the emergence of pain (Kubacey et al. 2012).

5.4.8 Various

Vitexin and isovitexin, isolated from mung beans (*Vigna radiata*), were tested for their ability to inhibit tyrosinase (see Fig. 6). Tyrosinase is an essential enzyme in the melanin synthesis and therefore applicable in cosmetic industry for

hyperpigmentation and as whitening agent. In an in vitro assay vitexin and isovitexin were found to inhibit tyrosinase with an IC_{50} of 6.3 and 5.6 mg/ml, respectively (Xiao 2017). The sprouts of buckwheat (*Fagopyrum esculentum*) showed promising results in an in vivo study against stress in mice. The sprouts of this grain are rich in rutin, orientin, isoorientin, vitexin, and isovitexin. Mice were intragastrically given a 100 mg/kg buckwheat sprout solution and then exposed to stress (limited food and immobilization). The control group (no buckwheat in the solution) showed elevated corticosterone, glucose blood level, hyperlipidemic and thiobarbituric acid reactive substance values. The last substance is assayed in order to gain insight into the oxidative damage effect of stress. All these factors were downregulated by buckwheat flavonoids and therefore are a promising candidate for antistress treatment. Furthermore, plasma concentrations were determined for each CGF in buckwheat sprouts and it was found that 2 h after oral administration all CGF were intact (Xiao 2017). Isovitexin and vitexin were isolated from various plants of Argentina and tested for their ability to reduce oxidative stress (ROS) which was induced by gentamicin. Both CGF were able to inhibit the reactive oxygen species production in mononuclear leucocytes with 22.4% and 34.3% when 10 μM of the flavonoid was administered. In an additional experiment, IC_{50} values of ROS production were determined resulting values for isovitexin and vitexin of 75.36 and 22.7 μM, respectively. As reference substance vitamin C was tested and lead to an IC_{50} of 1.06 μM. The actual antistress experiment was only conducted with luteolin which was able to show a significant decrease of ROS compared to the control group. Luteolin was also able to reduce lipid peroxidation in this context. Furthermore, it was able to enhance the susceptibility of *Escherichia coli* and *Staphylococcus aureus* in combination with gentamicin (Bustos et al. 2018).

5.5 Benefits (Human Studies)

Increasing interest in flavonoids lead to an increasing number of research studies which step-by-step shed light on the metabolism and activities of this class of substances. Since not all flavonoids from one class behave in the same way in the body the clarification and testing of various flavonoids of one class are needed. Due to the fact that in vivo studies with humans are very limited, general conclusions are difficult to make. Especially the family of C-glycosylated flavonoids need a lot more attention. From the literature, it can be concluded that C-glycosylation is beneficial for the antioxidant and antidiabetic activity. In general, flavonoid glycosides are able to create higher plasma levels than their corresponding aglycones. The influence of the attached sugar has to be investigated very carefully in order to predict absorption and metabolism in vivo (Xiao 2017).

The bioactivities of CGF can be seen in subchapter 4 which presents some promising effects such as antidiabetic and anti-inflammatory action. However, until the application in humans against diabetes is feasible, a lot more research has to be done.

In plant derived food, CGF do not occur as singular phenolic compounds, but are embedded in a complex mixture including also other types of flavonoids except CGF.

There are some human studies of dietary flavonoids which have been comprehensively reviewed by Crozier et al. (2010) and Del Rio et al. (2013). Unfortunately, no CGF were specifically examined in these studies. Some data is available for CGF which only show the metabolization of CGF by intestinal bacteria. They are able to cleave the sugar moiety and release the pure aglycone. For instance, it was found that the *Lachnospiraceae* CG19-1 strain is able to metabolize the CGF vitexin to its aglycone apigenin. Also, the binding affinities of flavonoids to human serum albumin are documented in literature. For the bioactivity, human cell lines were utilized but no in vivo data for humans is available until now for CGF.

One aspect in literature has to be taken into account when dietary flavonoids are administered for boosting health. It is reported that flavonoids could be substrates for p-gp which leads to an efflux of these compounds back into the lumen of the gut. Therefore, no effect is exhibited by these administered flavonoids. Another aspect is the importance of p-gp in cancer chemotherapy. Overexpression of p-gp plays an important role in the development of cancer cell resistance. The working group around Di Pietro et al. tested various flavonoids as potential efflux pump inhibitors against various efflux transporters. The in silico predicted binding affinity for vitexin was so low that no further experiments were determined. But in the overall experiment it was shown that glycosylation of flavonoids is detrimental for the binding affinity to efflux transporters (Di Pietro et al. 2002).

5.6 Application in Food

Data on alterations of CGF in plant derived food during processing are quite limited. In rooibos (leaves and stems) a significant difference in unprocessed and fermented herbs can be observed. Fermentation leads to a severe loss of aspalathin which undergoes oxidative cyclisation to the corresponding flavanone C-glycosides. Further oxidation results in flavone C-glycosides (Courts and Williamson 2015; Hostetler et al. 2017). Oxidation is also a prerequisite in processed cocoa seeds to form C-glycosylated flavan-3-ols nonenzymatically. This process leads to a less bitter product as the puckering astringent taste of epicatechin and catechin is converted to a smooth astringent sensation without exhibiting a bitter taste (Stark and Hofmann 2006; Courts and Williamson 2015). High temperature applied for sterilization and preparation of food products indicate a high probability of such nonenzymatically generated C-glycosides, which could be present in the human diet. Furthermore, undergoing a Wessely-Moser rearrangement isomerization concerning C-6 or C-8 position of bound monosaccharides under cooking conditions could take place (Courts and Williamson 2015). For kudzu roots extracts, containing significant amounts of the isoflavonoid C-glycoside puerarin, cooking in beef patties did not lead to a relevant loss of puerarin content, indicating stability of this isoflavonoid C-glycoside during cooking (Kumari et al. 2015). Of course, as with water soluble glycosides cooking will leach out these compounds from the food material, however,

generally a higher stability compared to their O-glycosidic counterparts can be expected.

5.7 Safety: Toxicity and Side Effects

Studies on antinutritional effects have been performed for pearl millet (*Pennisetum glaucum*) (Gaitan et al. 1995; Boncompagni et al. 2018). Aside from the most relevant antinutrient compound, phytate, CGF were reported to have unwanted goitrogenic effects. The compounds in question are vitexin, glucosyl vitexin, and glucosyl orientin. Epidemiologic studies indicate that endemic goiter in rural areas of Africa and Asia might be related to a diet based on pearl millet as staple food (Boncompagni et al. 2018). In vivo antithyroid activity by purified vitexin could be shown in rats at 80 μM test concentration, 20 μM was not active (Gaitan et al. 1995), supporting this assumption.

In a study of Fantoukh et al. (2019), the influence of a methanolic extract of unfermented rooibos tea and selected chemicals of this plant material including the C-glycosyldihydrochalcones aspalathin and nothofagin as well as the C-glycosylflavones vitexin, isovitexin, isoorientin, and the C-glycosylflavanone eriodictyol-6-C-glucoside on interaction with cytochrome P450 enzymes (CYP), pregnane-X-receptor (PXR) and p-gp were investigated. Whereas the results obtained with the methanolic extract are only of limited relevance for infusions made for food purposes, the activities of pure compounds could give some indications of potential interactions with simultaneously taken pharmaceutical drugs or other food phytochemicals. Most prominent inhibitory effects were found for isovitexin on CYP3A4 (IC_{50} 3.4 μM) and for vitexin on CYP2C9 (IC_{50} 8.0 μM). Aspalathin and nothofagin exhibited only a moderate activation of PXR, associated with an increased mRNA expression of CYP3A4 and CYP1A2.

5.8 Marketed Products

There are no food products on the market specifically due to CGF content. Compared to food products made from plain flour, higher contents of CGF can be found in corresponding food products prepared from whole meal flour (Bucar, unpublished data; Hirawan and Beta 2011). However, these products are generally richer in plant phenolics. Rooibos herbal teas might serve as an example which focuses on a CGF (aspalathin) as active ingredient (Courts and Williamson 2015; Hostetler et al. 2017).

5.9 Patents

One Chinese patent describes the preparation of a C-glycosylflavones extract and a preparation method thereof, among others bamboo leaves are mentioned as source as well as blueberry without defined species (CN 103159750 A 20130619). Several

patents have been filed for rooibos and aspalathin, i.e., an antidiabetic extract from rooibos (US 8877717 B2 20141104), an aspalathin-like dihydrochalcone, extracts from unfermented rooibos and the treatment of the central nervous system (EP 2053050 A1 20090429), an antidiabetic extract of rooibos (WO 2008110551 A1 20080918), related to that a product which contains aspalathin and acts as a pancreatic β cell insulin secretion and tissue glucose promoting agent (JP 2008214274 A 20080918), an anticancer agent containing aspalathin (JP 2007197409 A 20070809), and a rooibos extract with increased aspalathin content (WO 2006081989 A1 20060810). Numerous patents have been filed for puerarin containing plant materials, and this is true also for vitexin and vitexin containing materials. To mention a few, a process to extract passion fruit skin for a phenolic rich extract contain CGF like vitexin and orientin was described (BR 102016030133 A2 20180717), similarly taro bean leaves (CN 107281258 A 20171024) as well as common buckwheat husk (CN 105859701 A 20160817) or peeled mung bean (KR 2009045964 A 20090511) can serve as source for CGF.

5.10 Perspectives

The major class of C-glycosylflavonoids in food plants is represented by flavones, in addition dihydrochalcones and some selected examples from C-glycosylisoflavones can be found. Citrus fruits can be considered as a major source of C-glycosylflavones, whereas relatively low amounts have been found in cereals with exception of millet seeds and carob seed germ flour. A rich source of C-glycosylated dihydrochalcones are tomatoes, as well as rooibos and honeybush herbal teas, and the most common C-glycosylisoflavone puerarin is mainly consumed via kudzu roots.

Due to their higher chemical stability in terms of hydrolysis during cooking and also after ingestion, they can be considered as a specific group within flavonoids. Their metabolic fate is clearly different from O-glycosidic flavonoids with absorption of intact glycosides, followed by phase II metabolization. However, also deglycosylation by gut microbiota and degradation of aglycones to compounds like (hydroxy) phenylpropionic acids have been recognized. The catabolic potential of gut microbiota for C-glycosylflavonoids seems to be still underestimated with insufficient data on microbial community – catabolic activity correlations. It can also be recognized that only limited data on the actual daily intake of C-glycosylflavonoids including information on content in fresh and processed food are available. This is also important for considering the appropriate doses for in vitro and in vivo studies which have shown a wide range of bioactivities of C-glycosylflavonoids.

5.11 Cross-References

► Biflavonoids and Oligomeric Flavonoids from Food
► Chalcones in Diets

▶ *Citrus* Flavanones
▶ Dietary Flavonols and O-Glycosides
▶ Prenylated Flavonoids in Food
▶ Soy Isoflavones
▶ Tea Catechins
▶ Theaflavins, Thearubigins, and Theasinensins

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Biflavonoids and Oligomeric Flavonoids from Food

6

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Contents

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Abstract

Biflavonoids and oligomeric flavonoids are the representatives of flavonoids from the diet, which can be classified into several subclasses according to the essential chemical structures. Proanthocyanidin and biflavonoid are two types of representative compounds that are widely distributed in various foods. Although the previous studies have verified that the increase of the polymerization degree of these molecules usually lowered the *in vivo* bioavailability, the *in vivo* metabolism of these compounds still remained indeterminate. Some of the most commonly reported *in vitro* activities of biflavonoids and oligomeric flavonoids were summarized subsequently. And the applications of biflavonoids and oligomeric flavonoids in several processed foods were elucidated because the chemical change during the processing of these compounds can affect the taste and color of the foods. Moreover, the effect on the prevention and relief of some chronic diseases, as well as the uncertain side effects and toxicity of the commercial products being popular on the market, were summarized and analyzed. In addition, the effects of biflavonoids and oligomeric flavonoids on public health, the patents that related to the extract and preparation method, and prospectives and recommendations of their applications were proposed.

Keywords

Diet · Biflavonoids · Oligomeric flavonoids · Proanthocyanidin · Bioavailability · Metabolism · Bioactivities

6.1 Introduction

Biflavonoids and oligomeric flavonoids are kinds of fascinating molecules that are a major class of flavonoids ingested from food. And they are always found in the great consumption of food, such as apple, grape, berry, cereal, beans, etc. Moreover, the eye-catching color and unpredictable astringency of red wine, black tea, and juice are inseparable from these molecules. Most importantly, these compounds have been proved to be beneficial to our health (Bhagwat and Haytowitz 2015; Ou and Gu 2014). These special characteristics of biflavonoids and oligomeric flavonoids inspire us to investigate them and develop new products to benefit human beings.

The concepts of biflavonoid and oligomeric flavonoid are easily confused with that of flavonoid, polyphenol, and tannin, because these compounds are overloppingly defined owing to their similar chemical structures, bioactivities, and distribution in food. In fact, their phytochemicals are essentially different. It is also difficult for nonprofessionals to distinguish the names of the chemicals and

structures of flavonoid monomers, dimers, and polymers. In this chapter, a precise definition of biflavonoid and oligomeric flavonoid was put forward. Correspondingly, these natural compounds were classified according to their chemical structures and distribution in food.

It has been proved that the *in vivo* absorption and metabolism of the dietary phytochemical are closely related to their health promotion effects and possible potential side effects. Therefore, the bioavailability of these molecules after being ingested, the metabolic processes in the human body, and the interaction of these molecules with gut microflora are systematically investigated and summarized. Moreover, some excellent biological activities, such as the antioxidant activity, antiproliferative activity toward cancer cells, anti-inflammatory, antimicrobial activity, and lipase inhibitory activity of biflavonoids and oligomeric flavonoids, are introduced. Several recognized benefits of these compounds are discussed subsequently, including cardiovascular protection, losing weight, and prevention of neurodegenerative diseases.

The chemical changes of these molecules during food processing dramatically affect the color, aroma, and flavor of foods. Thus, biflavonoids and oligomeric flavonoids were also introduced as color, aroma, and/or flavor modifiers in tea, red wine, juice, cocoa, etc. Although the benefit of biflavonoids and oligomeric flavonoids outweighs their risk, the toxicity and side effects of these dietary phytochemicals should be considered. The biosafety of biflavonoids and oligomeric flavonoids in food is the foundation of the marketed products. Moreover, the patents within two decades that related to these compounds, the framework of biflavonoids and oligomeric flavonoids in extraction technology, and the functional application were presented. In the end, the future outlook and development directions of biflavonoids and oligomeric flavonoids in the field of food were respectively described and recommended.

6.2 Bioactive Constituents

Biflavonoids and oligomeric flavonoids in food have gained wide attention of researchers over decades due to their health promotion effects and fascinated flavors. Biflavonoid and oligomeric flavonoid are plant secondary metabolites and they both belong to flavonoid polymer. As a subgroup of the flavonoid family, they also have the C_6—C_3—C_6 basic skeleton in their monomers. Strictly, oligomeric flavonoid contains flavonoid dimer, trimer, and polymer with limited degrees of polymerization. Since the special chemical structure and bioactivity of some flavonoid dimers have been reported, flavonoid dimer is generally called biflavonoid as an individual concept distinguishing from oligomeric flavonoid. Biflavonoid and oligomeric flavonoid are a class of functional components which exist in foods and beverages. These compound can be classified into several major categories base on their chemical structure of their flavonoid monomer, for example, proanthocyanidin, which is formed by the polymerization of flavone-3-ol monomer, and biflavonoid, which are flavone or flavanone dimers (also called biflavone). Moreover, these

molecules can be changed during food processing and storage, and some new biflavonoids and oligomeric flavonoids are also generated (Tanaka et al. 2010).

As mass consumption beverages, tea, red wine, and cocoa are all rich in proanthocyanidin. Apple and its processing products are the most important source of dietary proanthocyanidin. This compound is also abundant in many berries, beans, grain, chickpeas, nuts, and cinnamon, serving as dietary proanthocyanidin (Bhagwat and Haytowitz 2015; Bittner et al. 2013; Hammerstone et al. 2000; Santos-Buelga and Scalbert 2000). Some species of *Garcinia*, a class of fruits cultivated in the tropics, are also favored by consumers because of its abundant biflavonoid. *Ginkgo biloba* is also a very famous edible plant with massive biflavonoids. Moreover, biflavonoids and oligomeric flavonoids have also been found in some functional foods and herbs (Gontijo et al. 2017; Latief et al. 2015; Lu et al. 2004; Nkengfack et al. 2002; Yan et al. 2017).

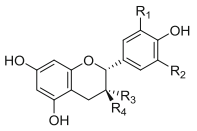
6.2.1 Proanthocyanidin

Proanthocyanidin is the largest class of flavonoid polymer and found in various foods, representing a major class of flavonoids ingested from the diets (Neilson et al. 2016; Ou and Gu 2014). Chemically, proanthocyanidin is a polymer formed by the condensation of flavan-3-ol. And the basic units usually are catechins and epicatechins. Proanthocyanidins can depolymerize to anthocyanidins under oxidative conditions, and their chemical structures depend upon the polymerization number, linkage ways, and substitution pattern of the basic units. There are three subgroups of proanthocyanidins, i.e., procyanidin, prodelphinidin, and propelargonidin (Fig. 1). Two types of linkage ways are named as type A and type B of the interflavanoid bonds in proanthocyanidin. A-type linkage has a less common feature in proanthocyanidins with C—C and C—O—C interflavanoid bonds, while B-type linkage is most common with the C—C bonds only (Fig. 1). Oligomeric proanthocyanidins strictly refer to dimer and trimer polymerizations of basic units. But there is also a denotation that proanthocyanidin with a degree of polymerization less than 10 is called oligomer. The A-type and B-type linkages simultaneously occurr in one molecular, and the gallic acid ester or glycoside substitute usually appears in the oligomeric structure. Some proanthocyanidins, never contain sugar residues, are called condensed tannins (Bhagwat and Haytowitz 2015; Hümmer and Schreier 2008; Smith 2000).

As one of the most famous class of flavonoid polymers, proanthocyanidins are essential functional components that naturally occurr in polyphenolic compounds, being widely available in fruits, berries, nuts, beans, grains, and bark. Meanwhile, they are determinants of flavor and astringency in teas, wines, and fruit juices. The US Department of Agriculture (USDA) and European Food Information Resource (EuroFIR) periodically report the proanthocyanidin content in some functional foods (www.usda.gov; www.eurofir.org). The main proanthocyanidins in some selected functional foods were listed in Table 1.

A

Subgroup	monomer	Subsitution pattern			
		R_1	R_2	R_3	R_4
Procyanidin	Catechin	OH	H	H	OH
	Epicatechin	OH	H	OH	H
Prodelphinidin	Gallocatechin	OH	OH	H	OH
	Epigallocatechin	OH	OH	OH	H
Propelargonidin	Afzelechin	H	H	H	OH
	Epiafzelchin	H	H	OH	H



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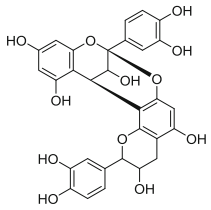

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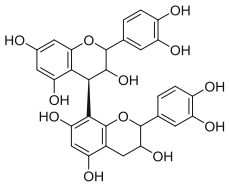

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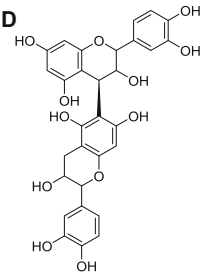


Fig. 1 Chemical structure of proanthocyanidin basic units and the main linkage ways. (**a**) Structure of common flavan-3-ols and substitution patterns found in proanthocyanidins. (**b**) A-type linkage, (**c**) B-type 4 → 8 linkage, and (**d**) type 4 → 6 linkage

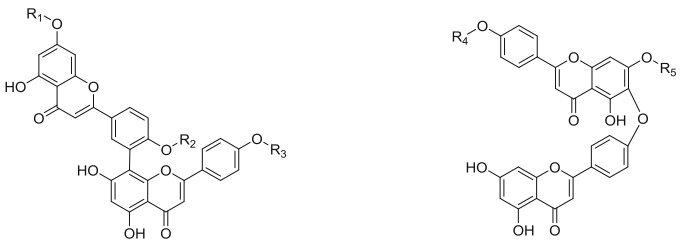
6.2.2 Biflavonoid

Biflavonoid is a class of flavonoid dimer that is differing from proanthocyanidins, which is primarily formed by flavone or flavanone constitutional units. And the units could be interconnected by C—C or C—O—C interflavanoid bonds, involving A-, B-, or C-rings of the monomer. And more than one interflavanoid bond of the monomer is connected with biflavonoids. Normally, the chemical discrimination between flavone, flavanone, and flavonol monomer is necessary. However, the interflavanoid bond of biflavonoid occurs in the C-ring of the monomer and trimers constituted by flavone or flavanone, occasionally leading to a very difficult distinguishment between biflavone and biflavanone. The most common substituents of biflavonoid molecules are glycoside and methoxy (Fig. 2) (Gontijo et al. 2017).

As a functional component, biflavonoid is only found in several common foods, such as *Garcinia*, *G. biloba*, and onion (Gontijo et al. 2017; Ly et al. 2005; Mohamed 2008). *Garcinia* is a genus of flowering plant in the family Clusiaceae native to Asia, America, Australia, tropical and southern Africa, and Polynesia. And there are nearly 400 acknowledged species all over the world. Most of them are recognized and used locally, only a few are well known globally. *G. mangostana* (also called purple mangosteen) and *G. madruno* are the most famous species of *Garcinia*.

Table 1 Procyanidins in some selected functional foods

Names	Chemical structure	Source of foods
Procyanidin A1	Epicatechin-(2β→7,4β→8)-catechin	Areca nut, buckwheat, cranberry, litchi, mulberry, peanut, tea
Procyanidin A2	Epicatechin-(2β→7,4β→8)-epicatechin	Apple, avocado, bilberry, cranberry, cocoa, grape, black soybean, litchi, peanut, red chicory, saskatoon berry, tea
Procyanidin B1	Epicatechin-(4β→8)-catechin	Apple, almond, beans, blueberry, bilberry, cocoa, grape, kiwifruit, olive, pear, pistachio, strawberry, tea
Procyanidin B2	Epicatechin-(4β→8)-epicatechin	Apple, almond, blueberry, bilberry, cocoa, grape, hawthorn berry, kiwifruit, pear
Procyanidin B3	Catechin-(4α→8)-catechin	Almond, beans, bilberry, buckwheat, grape, kiwifruit, peanut, strawberry
Procyanidin B4	Catechin-(4α→8)-epicatechin	Apple, grape
Procyanidin B5	Epicatechin-(4β→6)-epicatechin	Apple, almond, black soybean, buckwheat, cocoa
Procyanidin B6	Catechin-(4α→6)-catechin	Beans, hazelnut, pecan nut, walnut
Procyanidin B7	Catechin-(4α→6)-epicatechin	Almond, beans, grape, peanut
Procyanidin C1	Epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin	Apple, almond, black soybean, cinnamon, grape, hawthorn berry
Procyanidin C2	Catechin-(4α→8)-catechin-(4α→8)-catechin	Beans, buckwheat, hazelnut, grape, strawberry



Amentoflavone: R1 = H, R2 = H, R3 = H.
Isoginkgetin: R1 = H, R2 = CH_3, R3 = CH_3.

Isocryptomerin: R4 = H, R5 = CH_3.
Cryptomerin A: R4 = CH_3, R5 = H

Fig. 2 Chemical structure of biflavonoids

The white and fragrant botanically endocarps are edible, and the exocarp of purple mangosteen is used as a spice. More than 30 kinds of biflavonoid have been identified from *Garcinia*. Many of the species are locally used as traditional medicine. Some representative biflavonoids were isolated from *Garcinia* plants, such as amentoflavone and GB series. The first biflavonoid, ginkgetin, is isolated from *G.*

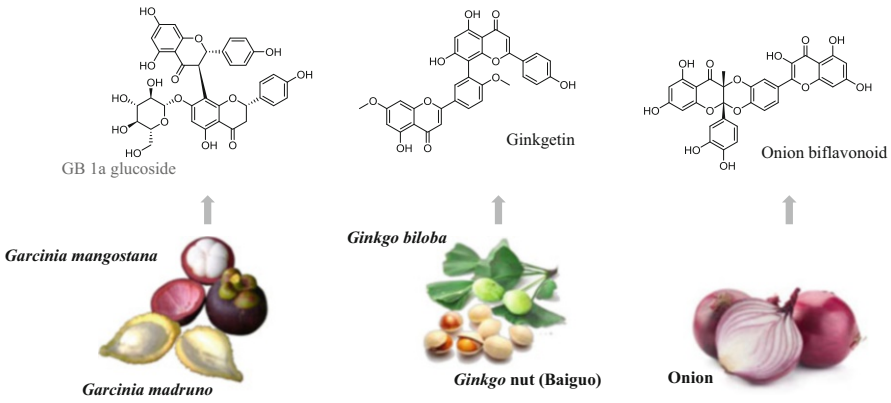


Fig. 3 Representative biflavonoids in *Garcinia*, *Ginkgo biloba*, and onion, respectively

biloba. Being the only living species in the division of Ginkgophyta, the fruit (nut) of *G. biloba* is edible, and the leaves can be processed into medical oral liquids. Many people believed that the proper utilization of *Ginkgo* nut and leaves is good for the health. Moreover, other famous biflavonoids like isoginkgetin and sciadopytisin also can be found in *G. biloba*. As an important flavonoid source of vegetables, onions are cultivated and used all around the world. It has been proved that some biflavonoids can be isolated and identified from outer layers of the onion and some bioactivities of the onion biflavonoids have been also investigated (Fig. 3). In addition to the above three edible plants, biflavonoids have also been found in some other plants with medicinal or health functions, such as *Semecarpus anacardium*, *Sorbus domestica*, *Coreopsis tinctorial*, and so on (Murthy 1986; Termentzi et al. 2009; Yan et al. 2017).

6.2.3 Other Natural Biflavonoid

Other flavonoid monomers, such as chalcone, dihydrochalcone, xanthone, aurone and isoflavone, can also naturally form polymers, especially dimers. But these compounds are very rare in diets. Most of them are found in medicinal plants, and few are identified in some special foods. For instance, humulusol (a kind of chalcone dimer), which was found in *Humulus lupulus*, can be used as an important flavoring agent for beer (Yu et al. 2014). Previous studies have showed that some species of *Garcinia* contain xanthone dimers, but most of them have been found in the bark, and just seven xanthone dimers have been isolated from exocarp of *G. mangostana* (Liu et al. 2016). As for isoflavone dimer, it has been found in *Tadehagi triquetrum* which is a traditional herb that can be cooked with foods (Xiang et al. 2005). Beyond that, there exists a kind of special biflavonoid that two flavonoid monomers co-exist in one molecule without a direct link. For example, the biginkgoside series have been found in *G. biloba* leaves, and their basic chemical structure is that two

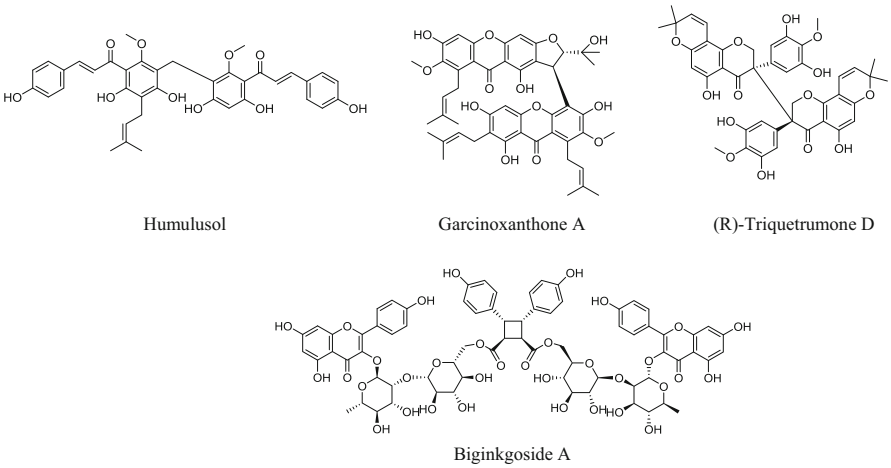


Fig. 4 Representative chalcone dimer, humulusol, xanthone dimer, garcinoxanthone A, isoflavone dimer, (R)-triquetrumone D, as well as a special biflavonoid, biginkgoside A

flavonoid glycoside moieties respectively connect to a cyclobutanedicarboxylic acid fragment (Fig. 4) (Ma et al. 2016).

6.2.4 Biflavonoids and Oligomeric Flavonoids Produced During Food Processing

During food processing and storage, some flavonoids with active chemical properties are often polymerized. Meanwhile, the original flavonoid polymers in food also undergo some reactions to generate new molecules or change the degree of polymerization. The above changes always occur in tea, wine, juice, and cocoa.

Oxidation is the most common reaction involved in the production of new biflavonoids or oligomeric flavonoids. On one hand, the oxidation can build the new interflavanoid at the cost of two flavonoid monomers. On the other hand, it modifies the chemical structure of the monomer molecules. For example, theaflavin and theasinensin are the representative oxidative biflavonoids in tea. They are all generated by the enzymatic oxidation of epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG). Dehydrodicatechin is the first reported biflavonoid produced by oxidation in tea, which is generated by catechin dimers and trimers. EGC and its quinone can condense to dehydrotheasinensin or proepitheaflagallin. The same condensation is also occurred in GC. Moreover, the secondary oligomeric flavonoids can also produce more complex flavonoid polymers, such as bistheaflavins, dehydrotheaflavin, and theanaphthoquinone.

There are also other small-molecule compounds that would be part of the flavonoid condensation. Aldehydes are always linked to oligomeric flavonoids

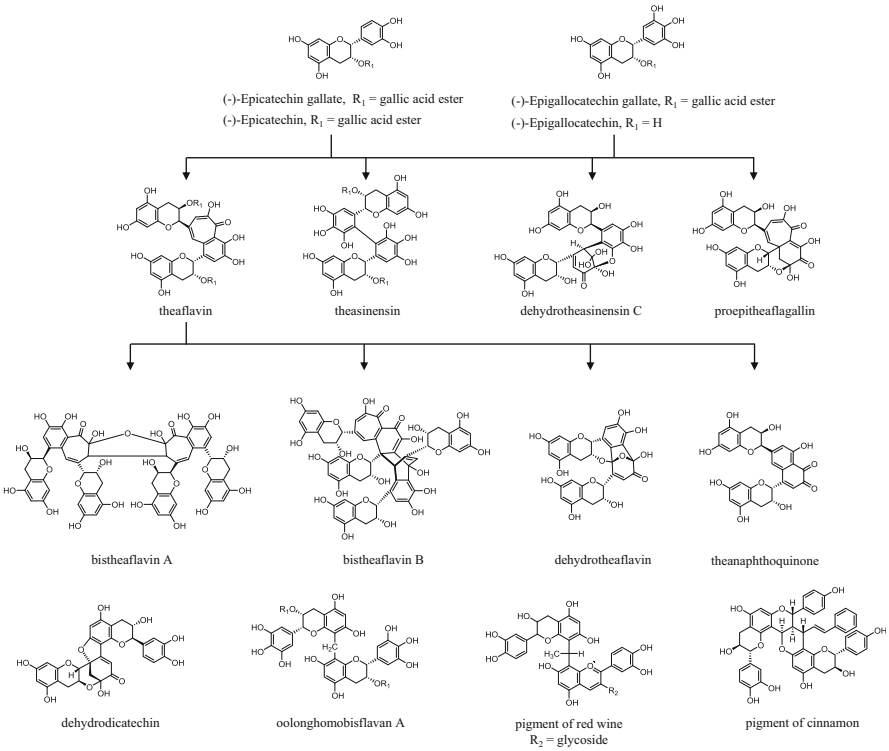


Fig. 5 Representative secondary oligomeric. Theaflavin, theasinensin, dehydrotheasinensin C, and proepitheaflagallin are produced by the oxidative condensation of epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin. Two bistheaflavins, dehydrotheaflavin and theanaphthoquinone, are produced by the oxidative condensation of theaflavin. Dehydrodicatechin is oxidized from catechin dimers and trimers. Oolonghomobisflavan A and pigment of red wine are two classes of biflavonoids connected by aldehyde. Pigment of cinnamon is procyanidin-cinnamaldehyde conjugate

during the storage of wine and the processing of tea. Oligomeric proanthocyanidin of persimmon fruits can be connected by acetaldehyde and results in insolubly condensed tannins with a high degree of polymerization. The reaction causes the astringency of the persimmon to decrease. Cinnamaldehyde also can be reacted with procyanidin to generate a dimeric product which is a reddish-brown pigment in cinnamon bark. Furthermore, the changes of the polymerization degree of procyanidin always occur in processing of juice (Fig. 5) (Alcalde-Eon et al. 2006; Tanaka et al. 2010).

Biflavonoid and oligomeric flavonoids are a large class of naturally functional components with widely distributed and diverse structures. Predictably, more and more biflavonoid and oligomeric flavonoid molecules will appear on our tables along with the development of food science and natural product chemistry.

6.3 Bioavailability and Metabolism

The metabolism and pharmacokinetics of biflavonoids and oligomeric flavonoids in foods are significantly helpful to understand the effects of these compounds on human. Bioavailability refers to the proportion of biflavonoids and oligomeric flavonoids that can be absorbed by the whole body. The bioavailability and metabolism of flavonoids are affected by many factors, including individual differences (i.e., sex, age, genotype, gut microbiota), as well as growing environment (i.e., region, climate, temperature), processing (i.e., baking, frying), storage and matrix (i.e., water, fiber) of food, etc.

In order to study the bioavailability and metabolism of biflavonoids and oligomeric flavonoids in human bodies, molecular biological and biochemical techniques are always utilized. Recently, metabolomics based on nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) have become an effective tool to analyze flavonoid metabolites, which helps us understand more about the flavonoid metabolism *in vivo* (Yonekura-Sakakibara et al. 2019). To understand the absorption and metabolism of biflavonoids and oligomeric flavonoids in foods, it is necessary to study various metabolites of biflavonoids and oligomeric flavonoids *in vivo* and identify the physiologically active ingredients. Herein, the recent progress of the bioavailability and metabolism of dietary biflavonoids and oligomeric flavonoids was summarized.

6.3.1 Proanthocyanidin

Proanthocyanidin is one of the most abundant biflavonoids and oligomeric flavonoids in fruits and vegetables. The bioavailability of proanthocyanidins is closely related to the degree of polymerization and molecular weight. Generally, the absorption ratios of proanthocyanidins are negatively related to the degree of polymerization and molecular weight (Ou and Gu 2014), and the absorption ratio of monomers may be 100-fold higher than that of dimers (Choy and Waterhouse 2014). Some monomers and dimers can be directly absorbed by the small intestine and found in plasma. Trimer and tetramer of proanthocyanidins are absorbed lower than dimers. If the degree of polymerization is higher than four, the large proanthocyanidins molecule may be unabsorbable due to the gut barrier (Ou and Gu 2014). Also, the absorption sites of proanthocyanidins are varied with the molecule weight and structure (Tao et al. 2019). The monomers are mainly absorbed by the proximal gut, while the oligomers and polymers have to be fermented by the gut microflora, and the fermentation metabolites are absorbed by the gastrointestinal tract (Tao et al. 2019). Moreover, the state of proanthocyanidins may affect the absorption, and only the soluble state of proanthocyanidins can be absorbed by enterocyte surface (Ou and Gu 2014).

Most of the dietary proanthocyanidins are oligomeric and polymeric; therefore the bioavailability of proanthocyanidins is relatively low. GC-MS and high-

performance LC-MS are used to evaluate the levels of proanthocyanidins *in vivo*. Results showed that after drinking red wine for 1.4 h, the level of catechin within the volunteer bodies could reach to 76.7 nmol/L (Bell et al. 2000). After ingesting 387.58 μmol of epicatechin for 48 h, 9.55 μmol of epicatechin appeared in urine (Castello et al. 2018). Without the enzymolysis of deconjugating enzymes, EGCG was absorbed in a ratio of 0.32% in human plasma and 0.012% in fasted rats (Gan et al. 2018; Nakagawa and Miyazawa 1997; Tao et al. 2019). The digestion and absorption of proanthocyanidins *in vivo* can be divided into two parts. The first part happens in an acidic condition of the stomach, and the second part is in gut. Proanthocyanidins first digested in the oral cavity and stomach. α-Amylase and gastric fluid help the depolymerization of proanthocyanidins, and then depolymerized proanthocyanidins reach to the gut. Only a small part of proanthocyanidins can be directly absorbed by the proximal intestine. Besides, glucuronidation of proanthocyanidins happens at small intestine catalyzing by uridine 5′-diphosphate glucuronosyltransferases. Over 90% proanthocyanidins will reach the colon, where these proanthocyanidins will be fermented by gut microflora and metabolized by intestinal enzymes before absorption. More than 30 kinds of metabolites and metabolite derivatives have been identified as the products of the interaction between proanthocyanidins and gut microflora, including phenylvalerolactones and phenolic acids. These small metabolites can be absorbed by the gut (Choy and Waterhouse 2014; Smeriglio et al. 2017; Tao et al. 2019).

After absorption in the gut, proanthocyanidin metabolites will further be transported into the liver for further metabolism. Typically, these metabolic processes include sulfated, glucuronidated, methylated conjugates, etc. Sulfation and methylation are catalyzed by sulfotransferases and catechol-*O*-methyltransferase, respectively. After liver metabolism, these metabolites can be secreted back to gut by bile (Choy and Waterhouse 2014). Moreover, these new metabolites can be found in different organs, blood, and urine (Ou and Gu 2014). Only small amounts of monomer and dimer proanthocyanidins are detected in the blood. Methylated and glucuronidated metabolites seem to be the majority part of proanthocyanidin metabolites in the blood (Smeriglio et al. 2017). Moreover, if the degree of polymerization is less than three, these proanthocyanidins can pass the blood-brain barrier and may affect the nervous system (Tao et al. 2019).

However, the transporters of proanthocyanidins have not been identified in the gut yet. Proanthocyanidins are mainly absorbed by passive diffusion (Ou and Gu 2014). Therefore, although proanthocyanidins are very abundant in food, they are absorbed at a relatively low ratio. It is considered that long-term and low-dose uptake of proanthocyanidins may be beneficial for the gut absorption (Tao et al. 2019).

In addition, the interactions between proanthocyanidins and gut microflora are very important for bioavailability and metabolism of proanthocyanidins. Compared with their parent metabolites, many gut microflora-fermented metabolites exhibited higher activities and healthier benefits. Many studies have proved that proanthocyanidins can change the compositions and metabolic activities of gut microflora (Liu et al. 2017). Cranberry proanthocyanidins are reported to increase the proportion of health-benefit bacteria, *Akkermansia muciniphila*, in gut microflora

(Anhe et al. 2015). Also, other proanthocyanidins from grape seeds are reported to decrease Firmicutes/Bacteroidetes ratio to promote health level (Zhang et al. 2018). Through metabolomics methods, 2-(3,4-dihydroxyphenyl) acetic acid and 5-(3,4-dihydroxyphenyl)-γ-valerolactone are found as the major product metabolites of procyanidin dimers digested by human gut microflora. Other product metabolites, e.g., 1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl) propan-2-ol, phenylvaleric acids, 3-hydroxyphenylpropionic acid, monohydroxylated phenylvalerolactone, 3-hydroxyphenylacetic acid, and 4-hydroxyphenylacetic acid are also detected (Appeldoorn et al. 2009). Until now, the proanthocyanidin-gut microflora interaction and bioactive composition of proanthocyanidin metabolites still remain largely unknown. And more studies should be conducted to clarify the issues. Figure 6 summarized the proanthocyanidin dimer B1 metabolic pathway by gut microflora. Proanthocyanidins are firstly catabolized to cleave the interflavanoid bond and the C-ring. The A-ring oxidation, dehydroxylation, and β-oxidation are followed, and then proanthocyanidins are finally converted to small metabolites (Tao et al. 2019).

Proanthocyanidins also help to improve the metabolism of human and moderate balancing of hormones (Pascual-Serrano et al. 2017). Since proanthocyanidins have the ability to antioxidant and maintain the redox balance *in vivo*, they can inhibit lipid peroxidation and the activity of lipoxygenases (Smeriglio et al. 2017). In a recent study, rats fed of high-protein diets transport the undigested proteins to the colon and increase the concentrations of branched-chain fatty acids, ammonia, and hydrogen sulfide. While after feeding with proanthocyanidin-rich extract from avocado peel, the concentration of hydrogen sulfide is decreased, and the concentration of indole is increased. Moreover, the polyphenol extract from avocado peel can also counteract high-protein diets with changing gut microflora (Cires et al. 2019). Therefore, food proanthocyanidins also have some beneficial effects on endogenous metabolism, which is closely related to the effects of proanthocyanidins on diabetes, obesity, metabolic disorders, and cancers.

6.3.2 Other Biflavonoids and Oligomeric Flavonoids

Compared to proanthocyanidins, the bioavailability and metabolism of other biflavonoids and oligomeric flavonoids are less studied. It has been reported that the intraperitoneal administration of biflavonoids has better bioavailability than oral administration. The oral bioavailability is considered very low (Kim et al. 2008). Although the bioavailability and metabolism of biflavonoids are still unknown, they may undergo hydrolysis with the products of monomers, being metabolized as the same rules as monomers (Tabares-Guevara et al. 2017). Usually, oligomeric flavonoids can be hydrolyzed to monomers and dimers by gastric acid in the stomach (Kumar and Pandey 2013).

Biflavonoids can antioxidize and modify the metabolism of mice. There are three possible active ingredients of biflavonoids *in vivo*. The first ones are the biflavonoid and conjugated biflavonoids. The second is biflavonoid-derived monoflavonoids at

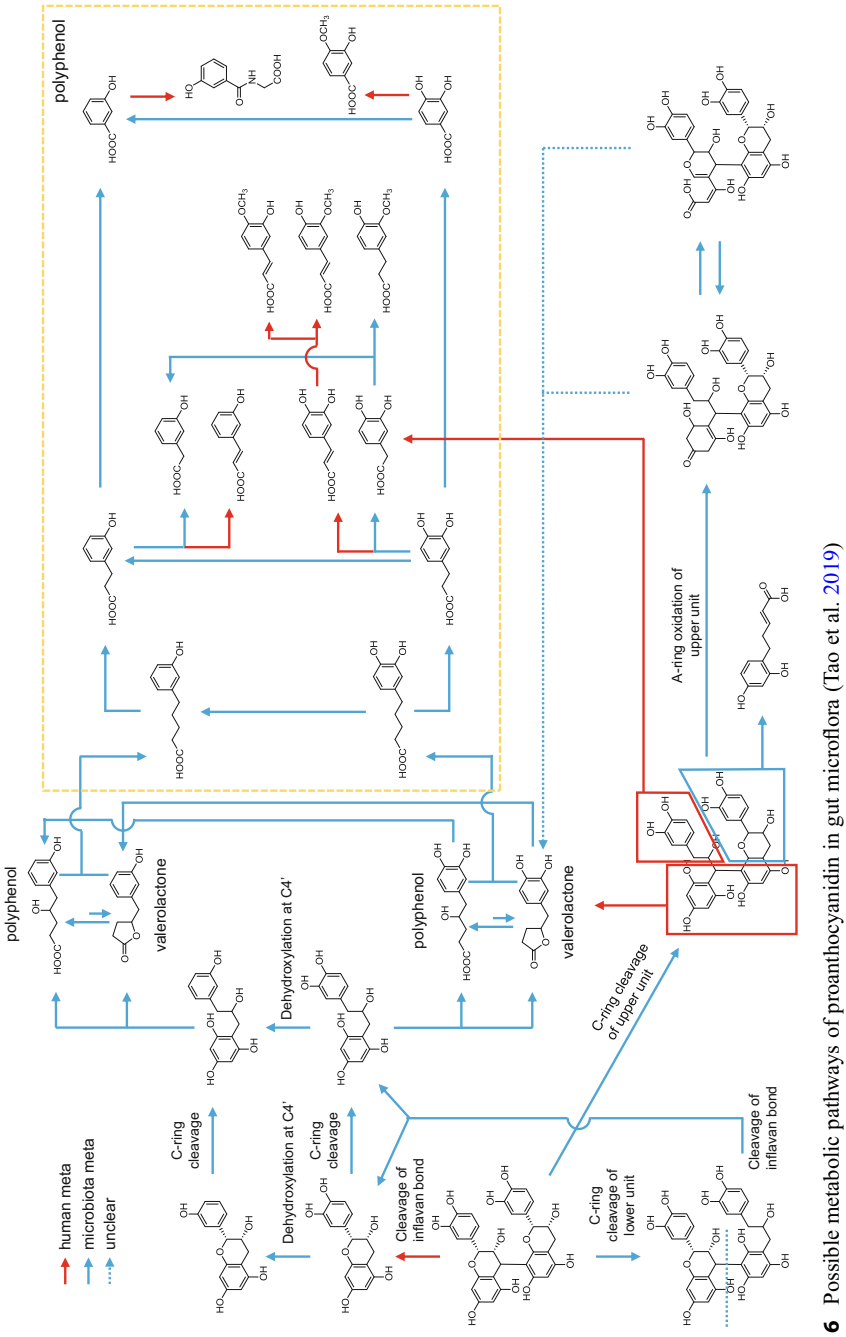


Fig. 6 Possible metabolic pathways of proanthocyanidin in gut microflora (Tao et al. 2019)

the forms of conjugation or unconjugation. The third possibilities are the microbial-derived catabolic products with conjugated and unconjugated forms (Tabares-Guevara et al. 2017).

Recently, chromatography-MS-based analysis techniques have been developed to study the metabolism and pharmacokinetics of biflavonoids and oligomeric flavonoids. Isoginkgetin, a natural biflavonoid proteasome inhibitor, has been successfully detected by LC-MS/MS-based method in rat plasma (Zhao et al. 2019a). LC-MS/MS-based method was also used to detect total oligomeric flavonoid fraction (Kandikattu et al. 2015). Besides, the pharmacokinetic of amentoflavone, a biflavonoid discovered in many plants, has also been investigated. Oral gavage and intravenous and intraperitoneal injection of amentoflavone were used to administrate rats, respectively. Conjugated metabolites of amentoflavone were detected in rat blood circulation system, and the results showed that much lower bioavailability of amentoflavone was found in oral gavage than the intraperitoneal injection (Yu et al. 2017). Chrysocauloflavone I, an uncommon biflavonoid, was performed in a pharmacokinetic experiment, and the results indicated after intravenous injection of chrysocauloflavone I in rats, it could be detected in all tissues, and the half-life of chrysocauloflavone I *in vivo* was about 85 min (Yang et al. 2016). Moreover, the total oligomeric flavonoids in *Cyperus rotundus* were found to restore the activity of glutamine synthetase, with catalyzing glutamate to glutamine (Sunil et al. 2011).

6.4 Bioactivities

Numerous studies have demonstrated that either crude or purified biflavonoids or oligomeric flavonoids, particularly from grape seed, cocoa, and tea, possess various excellent bioactivities, such as the antioxidant, antiproliferative, anti-inflammatory, and antimicrobial activities.

6.4.1 Antioxidant Activity

The antioxidant characteristics of extracts from grape seed, grape pomace, tea, and cocoa have been widely studied, including scavenging of free radicals, inhibition of lipid oxidation, reduction in hydroperoxide formation, and so on (Charradi et al. 2013). Grape seed extracts and cocoa by-products have shown high free radical scavenging activity via their high content of procyanidins (Cos et al. 2004; Lamuela-Raventós et al. 2005). There is a good coincidence between the degree of polymerization and the scavenging activity of procyanidin. Polymerization degree up to trimers will increase the free radical scavenging activity, while further polymerization will decrease this activity.

Procyanidins are often categorized as *in vivo* antioxidants since they have multi-hydroxyl groups in chemical structures, they can be preferentially oxidized. Procyanidins support the direct antioxidant abilities to balance both the steady-state concentration (production and metabolism) of cell oxidants and the occurrence of

oxidant-mediated events. Further, certain procyanidins can generate benefits in some pathological situations associated with high oxidant production, such as hypertension and cardiovascular disease, obesity, diabetes, etc. (Fraga and Oteiza 2011).

Proanthocyanidins extracted from grape seeds can provide more significant protection than vitamins C and E and β-carotene when similar doses are used, with lowering free radicals and free radical-induced lipid peroxidation and DNA damage in liver and brain tissues (Bagchi et al. 1997). *In vitro* studies have demonstrated that proanthocyanidins from grape seeds exhibit potent scavenger capacity for superoxide and hydroxyl radicals and can greatly inhibit the oxidation of low density lipoprotein (LDL) and lipid-containing membranes induced by metal ions or the other radical generators (Mazur et al. 1999). Furthermore, proanthocyanidins significantly improved the total antioxidative capacity of superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT) and reduced the malondialdehyde (MDA) levels compared with the oxidative damage mice model group through a meta-analysis (Smeriglio et al. 2017).

Amentoflavone can inhibit the production of superoxide anion and total reactive oxygen species (ROS) in phorbol 12-myristate 13-acetate-stimulated human neutrophils. It can also alleviate the oxidant hemolysis and lipid peroxidation in human erythrocytes induced by 2, 2′-azobis hydrochlorides. In addition, amentoflavone has been observed to suppress the production of nitric oxide (NO), prostaglandin E-2 (PGE-2), and the nuclear translocation of c-Fos, which is related to anti-inflammation (Yu et al. 2017). Another biflavonoid complex from *Garcinia kola*, named kolaviron, has stronger inhibition to H_2O_2 than the standard antioxidants, e.g., butylated hydroxyanisole and β-carotene, has the similar inhibition as α-tocopherol, and significantly scavenges superoxide generated by phenazine methosulfate NADH. Furthermore, kolaviron scavenges hydroxyl radicals via the significant inhibition of the oxidation of deoxyribose. In animal models, kolaviron reduces the background levels of protein oxidation marker in plasma, malondialdehyde in the membrane, and oxidative damage to DNA in the nucleus and mitochondria (Farombi and Owoeye 2011).

6.4.2 Antiproliferative Activity of Cancer Cells

The potential roles of proanthocyanidins and procyanidin in chemoprevention by dietary cranberry, grape seeds, and cocoa are gradually being emerged. As reported, the antiproliferative activity of proanthocyanidin-rich extracts from berries in multicancer cell lines (including oral cancer cell lines, e.g., KB and CAL-27; prostate cancer cell lines, e.g., RWPE-1, RWpe-2, and 22Rv1; lung cancer cells, e.g., HT-29 and A549; melanoma cells, e.g., A375 and Hs294T; colon cancer lines, e.g., HCT-116, and SW-620; breast cancer lines, e.g., MCF-7 and MDA-MB-435) has been elucidated (Neto 2007). Grape seed extracts have also exhibited antitumor properties to human colorectal carcinoma, head and neck squamous cell carcinoma, and prostate cancer cells (Baliga and Katiyar 2006). The treatment of human colonic cancer cells with procyanidin-enriched cocoa powder extracts reduced 70% of

cellular growth. In most studies, tea extracts were often used to treat rats or mice via oral administration (Santos-Buelga and Scalbert 2000).

On the base of studies on the working mechanisms of proanthocyanidins, various targeting molecules being potentially useful for the prevention or treatment of cancers have been identified. For instance, nuclear factor kappa-B (NF-κB) and its target proteins, mitogen-activated protein kinase (MAPK) signaling pathway, the P13K/Akt pathway, mitochondrial pathway, Wnt/β-catenin signaling channel, cell apoptosis, cytokines and matrix metalloproteinases, and cell cycle progression have discovered to involve in the antiproliferative functions of proanthocyanidins.

The anticancer properties of proanthocyanidins have also been illustrated by *in vivo* studies with animal models. Dietary feeding of grape seed proanthocyanidins inhibits UVB-induced photocarcinogenesis in SKH-1 hairless mice in terms of tumor incidence, multiplicity, and growth/size. It has been convinced that the protection from UVB-induced immunosuppression afforded by dietary grape seed proanthocyanidins may be associated with the protection from UVB-induced photocarcinogenesis. The treatment of grape seed proanthocyanidins inhibited tumor growth induced by subcutaneous inoculation of viable 4T1 murine mammary cancer cells in immunocompetent Balb/c mice with the extension of survival period. Dietary supplementation with grape seed proanthocyanidins was found to either inhibit the incidence of dimethylbenzanthracene-induced mammary tumor in Sprague-Dawley (SD) rats or inhibit azoxymethane-induced distal colon-crypt foci rats. Furthermore, adding grape seed proanthocyanidins to the diet of mice can significantly inhibit the growth of melanoma xenografts in nude mice (Nandakumar et al. 2008). Studies have shown that grape seed proanthocyanidins can inhibit the growth of A375 and Hs294T melanoma cells, which is related to the reduction of β-catenin melanoma cells.

Furthermore, several biflavonoids isolated from *Lonicera japonica* showed *in vitro* cytotoxic properties and proliferation inhibition against cell lines of colon, lung, kidney, ovary, and breast cancer (Gontijo et al. 2017). Ginkgetin is cytotoxic to human ovarian adenocarcinoma (OVCAR)-3 cells but enhances the proliferation of normal skin fibroblasts of human beings. Amentoflavone exerted obvious cytotoxic effects on cervical adenocarcinoma (HeLa) cells and MCF-7 cells (Kim et al. 2008). Some studies indicated that amentoflavone could inhibit the expression of fatty acid synthase (FASN) in human breast carcinoma SKBR3 cells containing epidermal growth factor receptor 2 (HER2). This inhibition decreased the translocation of sterol regulatory element-binding protein 1 (SREBP-1) in SKBR3 cells. In another experiment, amentoflavone was observed to increase the cleavage activity of caspase-3, to suppress SKBR3 cell activity, without affecting normal cell growth of FASN-none-expressed NIH-3T3 cells. Amentoflavone also could significantly inhibit solid tumor development that was induced by B16F-10 melanoma in C57BL/6 mice (Yu et al. 2017).

6.4.3 Anti-inflammatory

The biflavonoids, including amentoflavone, bilobetin, and ginkgetin, showed anti-allergic function. The other kinds of biflavonoids of ochnaflavone, ginkgetin, and isoginkgetin are generally reversible inhibitors of lymphocyte proliferation, e.g., T cell and B cell.

Amentoflavone showed potent anti-inflammatory activity *in vitro* and *in vivo*. Amentoflavone inhibited the increase of interleukin-1β (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α), and PGE 2 induced by phytohaemagglutinin in human peripheral blood mononuclear cells. Amentoflavone was also observed to suppress the production of NO, PEG-2, and the nuclear translocation of c-Fos, a subunit of activator protein (AP)-1. Amentoflavone possessed the anti-inflammatory activity of indomethacin or prednisolone against several animal models of acute inflammation, as well as the potent analgesic activity against acetic acid-induced writhings in mice (Kim et al. 2008).

Ginkgetin has inhibition effect of phospholipase A and it also can suppress pro-inflammatory genes in animal models of skin chronic inflammation. Ginkgetin was confirmed with inhibitory activity against adjuvant-induced arthritis with no severe side effects, such as the reducement of thymus and spleen weights. The *Garcinia* biflavonoids isolated from the leaves of *G. garderiana*, GB1 and GB2, showed *in vivo* anti-inflammatory activity against CGN-induced edema. Besides, amentoflavone, ginkgetin, and sciadopytisin showed anti-inflammatory activity against croton oil-induced ear edema (Gontijo et al. 2017; Loggia et al. 1996).

One anti-inflammatory mechanism of biflavonoids is that these compounds can affect the transcription proinflammatory molecules and biflavonoids were also can suppress the expression of the inducible nitric oxide synthase and COX-2 (Lee et al. 2006). The anti-inflammation activity of proanthocyanidins derived from grape seed was intensively studied. It was found the proanthocyanidins protected endotoxin-stimulated macrophages (RAW 264.7) from the overproduction of inflammatory mediators of NO, the production of PGE-1, and the expression of NO synthase. It was demonstrated that grape seed proanthocyanidin restrained the inflammatory response of activated neutrophil, thus preventing neutrophil adhesion and activation. *In vivo* studies have shown that high-fat diet (HFD) rats supplemented with grape seed procyanidins could attenuate the inflammatory markers in the liver, white adipose tissue, and circulation system, which were associated with the inhibition of the proinflammatory molecules C-reaction protein, IL-6 and TNF-α, and the enhancement of the anti-inflammatory cytokine adiponectin (Nowshehri et al. 2015).

6.4.4 Anti-obesity Activity

After treated with grape seed procyanidin extract, a significant decrease in body weight gain and the weight of white adipose tissue in HFD hamsters occurred. In

mice fed with HFD, supplements consisting of either cocoa flavanol extract or a flavanol fraction enriched with monomeric, oligomeric, or polymeric procyanidins prevented weight gain, increase of fat mass, impairment of glucose tolerance, and insulin resistance. A proanthocyanidin-rich fraction of *Cassia nomame* fruits was effective in preventing and ameliorating obesity.

Proanthocyanidins had inhibitory effects on digestive enzymes, such as lipase, α-amylase, and trypsin. Lipase-inhibiting effects had been shown by five biflavonoids from fruits of *Cassia nomame*. Procyanidin substantially inhibited pancreatic lipase activity and reduced triglyceride absorption. Furthermore, the inhibitory effects increase with the polymerization degree. Proanthocyanidin also showed inhibition effects of amylase and smaller oligosaccharide-digesting enzymes. The inhibition efficacy of proanthocyanidin extracted from some foods was in the order as follows: cranberry > grape > cocoa for α-amylase and grape > cranberry > cocoa for glucoamylase. The larger and the more complex the tannins are, the more effective inhibition of enzymes occurs. Proanthocyanidins isolated from *Manilkara zapota* (chiku) were also shown inhibition reaction of α-amylase and α-glucosidase. These *in vitro* results are supported by oral glucose tolerance tests in animals and by a limited number of human intervention studies. Two proanthocyanidin fractions from seed shell with different degree of polymerization both effectively suppressed the elevation of blood glucose from oral starch. Proanthocyanidins, furthermore, represented the inhibition activity on pancreatic proteases *in vitro* experiments.

Proanthocyanidins inhibit obesity through energy storage regulation including adipose hyperplasia and hypertrophy, glucose metabolism, and lipid metabolism. There were controversial results for the benefit from inhibition of adipogenesis by proanthocyanidins *in vitro* and *in vivo*. Proanthocyanidins have been demonstrated that increase glucose uptake in hepatocytes, adipocytes, and myotubes *in vitro*. Moreover, *in vivo* studies have proved that proanthocyanidins upregulated the expression of glucose transporter proteins-4 (GLUT-4) in adipose tissue and muscle and modulate GLUT-4 translocation to the plasma membrane. Animals' studies have shown a hypolipidemic effect of proanthocyanidins that also improved lipid homeostasis by increasing the reverse transport of cholesterol to the liver and its elimination via bile acids. Chronic treatment with grape seed procyanidin also reduced the hepatic steatosis induced by an HFD (Josepa Salvado et al. 2015).

6.4.5 Antimicrobial Activity

Flavonoids are small molecular secondary metabolites synthesized by plants with various biological activities. Their chemical, physical, and biochemical properties make them capable for the interaction of plants with other organisms like animals, microorganisms and other plants and also for their various reactions to environmental stresses (Mierziak et al. 2014). A biflavonoid class of flavonoids isolated from the plant of *Selaginella tamariscina* has antibacterial effects and combination effects of amentoflavone and conventional antibiotics (Hwang et al. 2013). The extracts from *Guazuma ulmifolia* Lam., mainly proanthocyanidins dimer B, have shown potential

as antimicrobial and antiprotozoal agents against a great number of diseases (Pereira et al. 2019). The antibacterial activity of proanthocyanidins originates from grape seed extracts (GSE) and has been investigated against different species and strains of Campylobacter (Silván et al. 2013). Moreover, a study has evaluated the potential antimicrobial use of GSE to inhibit the growth of *Alicyclobacillus acidoterrestris* cells and spores in apple juice during storage at 37 °C (Molva and Baysal 2015). Some naturally occurring and synthetic biflavonoids also show antibacterial, antifungal, and antiviral activities.

Amentoflavone was found having antifungal activity against several pathogenic fungal strains, including *Candida albicans*, *Saccharomyces cerevisiae*, and *Trichosporon beigelii*. In *Candida albicans*, it could stimulate the intracellular trehalose accumulation and disrupt the dimorphic transition, which meant a stress response to the component (Jung et al. 2006). Further research on its antifungal mechanism of *Candida albicans* suggested that this active phytochemical arrested cell cycles during the S-phase and inhibited cell proliferation and division. The anti-candida activity was proved to be related to apoptotic cell death, which may be associated with the mitochondrial dysfunction. Additionally, hydroxyl radicals induced by amentoflavone may play a significant role in apoptosis (Hwang et al. 2012).

6.5 Benefits

Dietary biflavonoids and oligomeric flavonoids have many health benefits. It is reported that the health benefits of biflavonoids and oligomeric flavonoids include reducing body weight, hypoglycemia, vasodilation, anti-inflammatory, antioxidation, antibacterial, neuroprotection, improving gut microflora, and so on. Because these are prominent benefits of biflavonoids and oligomeric flavonoids, they have been made into functional foods. In this part, we will systematically summarize the benefits of biflavonoids and oligomeric flavonoids in treatments of obesity, diabetes mellitus, neurological diseases, cancer, and cardiovascular disease (CVD).

6.5.1 Obesity and Diabetes Mellitus

Obesity and diabetes mellitus are globally enormous public health problems. In 2017, it was estimated that there were up to 425 million adults with diabetes (IDF Diabetes Atlas), and 650 million obese individuals in the world. Rises in prevalence of obesity coincide with the prevalence of type 2 diabetes mellitus which accounts for 90% of the total number of diabetic patients in almost all developed countries. Both are important causes of death and disability worldwide. People are more inclined to prevent, control, and alleviate these diseases through daily diet than medication (Bhupathiraju and Hu 2016).

Significant evidence suggests that proanthocyanidin-rich diets have the potentiality to protect against obesity and diabetes mellitus. Chronic procyanidin-rich apple

polyphenol administration significantly improved impaired glucose tolerance in high-normal and borderline subjects (Shoji et al. 2017). Improvements in insulin sensitivity and lower fasting blood glucose were observed in randomized controlled clinical trials that evaluated the therapeutic potential of cinnamon in healthy persons and type 2 diabetes patients (Yang and Chan 2017) and improved also significantly in overweight and obese adults consuming high-procyanidin cocoa for 12 weeks (Katz et al. 2011). And many clinical trials support the premise that cocoa can improve glycemic outcomes in healthy and overweight adults (Strat et al. 2016). Daily intake of chokeberry juice with rich procyanidin over a period of 3 months was effective in lowering fasting glucose levels in patients with non-insulin-dependent diabetes in a human intervention study (Kulling and Rawel 2008). Consumption of tea was inversely associated with the incidence of type 2 diabetes in a European population (Yang and Chan 2017). Grape seed extract significantly improved markers of inflammation and glycaemia and an oxidative stress marker obese type 2 diabetic subjects in a double-blinded randomized crossover trial (Kar et al. 2009).

Compared to the numerous clinical reports, the mechanism of these compounds' effect in the human body is not very clear. But there are some proposed mechanisms which were systematically analyzed. Procyanidins could lower hepatic glucose production by activating AMP-activated protein kinase (AMPK) and/or insulin-signaling pathways as well as involved in protecting pancreatic β cells from oxidative stress and promote insulin secretion and β-cell survival (Yang and Chan 2017). Procyanidins are also an inhibitor of the enzymes responsible for starch digestion, including α-amylase and α-glucosidase, which may respond to the suppression of the postprandial blood glucose (Kato 2019). Furthermore, some studies show that procyanidin modulates adipocyte processes such as lipolysis, lipid and glycogen synthesis, glucose uptake, and differentiation (Pinent et al. 2006). Proanthocyanidins have the ability to reduce body weight and fat storage *in vivo*. Firstly, proanthocyanidins can decrease the absorption of glucose and lipids by inhibiting the amylase and intestinal lipase (Blade et al. 2016). Proanthocyanidins can also decrease the absorption of bile acids and cholesterol (Tamura et al. 2013). Decreased absorption and digestion of dietary fat can alleviate hyperlipidemia. Secondly, proanthocyanidins can activate GLP-1/DPP4 (Gonzalez-Abuin et al. 2015) and regulate gastrointestinal tract-brain signals (Josepa Salvado et al. 2015). Thirdly, after absorption, proanthocyanidins will reach the adipose tissues, muscle, and liver, where proanthocyanidins can moderate gene and protein expression of lipid genes. Typically, proanthocyanidins inhibit lipogenesis as well as promote lipolysis, fatty acid oxidation, and mitochondrial biogenesis. It is reported that grape seed procyanidin extracts can reduce isoproterenol-stimulated lipolysis of 3T3-L1 cells (Moreno et al. 2003). Grape seed procyanidin extracts can also inhibit cholesterol esterase (Adisakwattana et al. 2010) and pancreatic α-amylase (Goncalves et al. 2011). Finally, proanthocyanidins can activate AMPK signaling pathway in the adipose tissues, muscle, and liver. Activated AMPK promotes thermogenesis, energy expenditure, and fatty acid oxidation and inhibits lipogenesis. However, some other studies find proanthocyanidins cannot reduce body weight (Casanova et al. 2014). Therefore, the anti-obesity effect of proanthocyanidins may rely on

dosage, administration duration, individual difference, and so on (Blade et al. 2016; Nie and Stürzenbaum 2019).

6.5.2 Neuroprotection

Neurodegenerative diseases which are the disturbance of cerebral redox homeostasis are incurable and debilitating conditions that are characterized by slowly progressive losses of neurons. Two neurodegenerative diseases, Alzheimer's disease (AD) and Parkinson's disease (PD), are the most prevalent. There are 46.8 million AD patients (in 2015) and 6.1 million PD individuals (in 2016) worldwide (Van Bulck et al. 2019). The intake of biflavonoids and oligomeric flavonoids as a class polyphenol which is easily obtained from natural foods was noticed to have a role in relieving and preventing the diseases (Del Rio et al. 2013). Long-term fruit juice with a high concentration of polyphenols consumption can provide protection against Alzheimer's disease (Dai et al. 2006). Procyanidin-rich grape juice consumption significantly enhanced the cognitive function relative to placebo-controlled elderly in a randomized control trial with 12 weeks. Grape juice supplementation with 12 weeks could improve the spatial memory and driving performance of middle-aged working mothers (Zhao et al. 2019b). Consumption cocoa flavanols based on proanthocyanidins have considerable benefits to cognitive function associated with healthy volunteers (Scholey et al. 2010). EGb® as a commercial *G. biloba* extract can significantly improve cognitive performance and social functioning regardless of patients with mild and severe Alzheimer's disease (Le Bars et al. 2002). The speed of response to the spatial working memory and immediate recognition tasks improved after supplementation with Enzogenol® which is a supplement of *Pinus radiata* bark extract (Pipingas et al. 2008). Intake of a commercial supplement Pycnogenol® from French maritime pine could improve spatial working memory of elderly individuals during a 3-month treatment period (Ryan et al. 2008).

Several published works have shown that these compounds can attenuate some pathophysiologic states such as oxidative stress, inflammation, neuron apoptosis, and the aggregation of Aβ peptide and Tau proteins (the pathological hallmarks of AD), but little is known of the molecular mechanism of these biological processes. Existing studies show that cAMP response element binding (CREB) has been identified to be essential in memory formation and consolidation. Silent information regulator 1 (SIRT1) as a potent therapeutic target for the prevention and treatment of AD could potentially modify the initiation and progression of AD by affecting multiple aspects of cortical and hippocampal neuron functions. And SIRT1 may regulate CREB activity via deacetylation in different tissues or organs. Procyanidins and its metabolites *in vivo* might involve in the phosphorylation of CREB against cognitive and memory impairment, then resulting in an upregulated expression of various genes which associate with the learning and memory. Moreover, procyanidin may be able to stimulate SIRT1-mediated signaling pathway to enhance the neural plasticity, improve cognitive function, and attenuate neurotoxicity caused by Aβ and Tau during AD (Zhao et al. 2019b). In addition, the biflavonoids from *G. biloba* can

significantly improve the coordinated motor ability of Parkinson's disease, via significantly inhibiting the expression of tyrosine hydroxylase in the substantia nigra and the activity of superoxide dismutase in the striatum (Kudolo et al. 2005). Although there has no report shows that procyanidins can reverse or cure neurodegenerative diseases, their neuroprotection capabilities could reduce the risk of the diseases and ameliorate relevant clinical symptoms as well as pathological features, consequently preventing and slowing down its progress.

6.5.3 Cancer

The global death toll due to cancers increased by 17.0%, to 8.8 million between 2005 and 2015 (Wang et al. 2016). It is an appealing proposal to increase the intake of polyphenols in the daily diet to prevent and treat cancer as well as relieve symptoms caused by cancer. Proanthocyanidins which are the most abundant classes of dietary polyphenols were reported to prevent cancer (Neilson et al. 2016).

Procyanidin dietary intake has been shown strongly inverse associations with colorectal cancer risk, based on data that examined six main classes of flavonoids and the risk of colorectal cancer from a national perspective case-control study in Scotland (Theodoratou et al. 2007). More than 50% reduction in the surface area of breast induration was recorded in 29.5% patients who were treated with the grape seed proanthocyanidin extract (IH636®) in 12-month double-blind, placebo-controlled, randomized phase II trial (Brooker et al. 2006). Seresis®, an oral supplement combination containing proanthocyanidins, was considered to have pharmacological prevention of skin cancer caused by UV (Greul et al. 2002). Increased tea consumption was associated with decreased numbers of breast cancer biomarkers. Green tea drinking reduces oxidative DNA damage, lipid peroxidation, and free radical generation in smokers and decreases in oxidative stress in nonsmokers. These indicators are beneficial for reducing cancer incidence (Dufresne and Farnworth 2001). Total flavonoids of diet, especially proanthocyanidins, showed suggestive protective associations with high-grade prostate cancer among men who were followed from 2 years after returning the 1999 survey. Of course, the association of higher dietary flavonoid intake with several healthier lifestyle factors cannot be overlooked (Wang et al. 2014). There is weak support for the protective effects of cocoa and chocolate against cancer risk and overall mortality based on limited epidemiologic evidence. But the data lack food frequency questionnaires that assess the intake of cocoa products (Maskarinec 2009).

The anticancer effects of proanthocyanidins own to their ability of antioxidation, anti-inflammatory, and targeting signaling pathway. ROS has been reported in many cancers. It is reported the grape seed proanthocyanidins have higher free radical scavenging capacity than vitamins C and E and can protect DNA from free radical damage (Bagchi et al. 2014). Grape seed proanthocyanidins can clear ROS to decrease the growth of colon cancer (Rauf et al. 2019). NF-κB signaling pathway is related to inflammatory, and activated NF-κB signaling pathway can promote cancer progression. Proanthocyanidins are reported to inhibit NF-κB signaling pathway as well as its targets in skin cancer cells (Meeran and Katiyar 2008).

Besides, cancer stem cells play very important roles in the development of cancers. Oligomeric proanthocyanidins are found to target the cancer stem-like cells of colorectal cancer and downregulate the markers of cancer stem cells. Also, oligomeric proanthocyanidins can inhibit Hippo signaling pathway (Toden et al. 2018). Although the intake of proanthocyanidins does not cure cancer, it is worthwhile to improve the diet without much cost in exchange for small prevention cancer benefits.

6.5.4 Cardiovascular Disease

The leading cause of noncommunicable disease death was CVD. There were 17.9 million died due to CVD in 2015 (Wang et al. 2016). Proanthocyanidins have been used in the treatment of CVD. The anti-CVD effects of proanthocyanidins mainly rely on its ability of antioxidation and anti-inflammatory. Reactive oxygen species (ROS) refer to unstable oxygen-containing chemical species. ROS can result in tissue injury and play a very important role in the development of CVD (Panth et al. 2016). Proanthocyanidins are naturally abundant antioxidant reagents. They can protect the body against oxidative stress. Moreover, the effects of the antioxidation of proanthocyanidins are related to their chain lengths. Long-chain proanthocyanidins have increased antioxidation activity than short-chain proanthocyanidins (Blade et al. 2016). Red wine contains abundant proanthocyanidins. It has been proved that moderate drinking of red wine can decrease the risk of coronary heart disease (Renaud and de Lorgeril 1992). Proanthocyanidins can significantly decrease the ROS such as hydroxyl and superoxide radicals *in vitro*. Blueberry supplementation to high-fat and cholesterol-feeding animals can help lowering blood pressure and relieving endothelial dysfunction (Rodriguez-Mateos et al. 2013). Anti-inflammatory effects of proanthocyanidins also contribute to their effects in treating CVD. Proanthocyanidins can change the expressions of inflammatory factors as well as target both MAPK and NF-κB signaling pathways (Mantena and Katiyar 2006). Arachidonic acid pathway is related to inflammatory reactions. Proanthocyanidins can modulate arachidonic acid pathway-related enzymes (Kruger et al. 2014; Blade et al. 2016).

Other biflavonoids and oligomeric flavonoids also have some effects of treating CVD. Kolaviron is a kind of biflavonoid extract which forms the seeds of *Garcinia kola*. It has cardioprotective effects. Kolaviron can inhibit lipopolysaccharide-induced inflammatory in vascular smooth muscle cells (Oyagbemi et al. 2016). Isoginkgetin and ginkgetin can inhibit NF-κB signaling pathways and have an ability to anti-inflammatory (Zhou et al. 2011).

6.6 Application in Food

Biflavonoids and oligomeric flavonoids are widely distributed in processed foods, such as tea, red wine, apples, and cocoa. These compounds can affect the color, aroma, and flavor of foods, especially contributing to less sweetness, more astringency, and bitterness. And the chemical change of natural biflavonoids and

oligomeric flavonoids during the food processing or storage will significantly affect the quality and nutrition of the foods. Therefore the changes of biflavonoids and oligomeric flavonoids become research focus.

During processing, the content, polymerization degree, and molecular structure of biflavonoid and oligomeric flavonoids are changing, which is a complicated process. Some changes in biflavonoids and oligomeric flavonoids are essential for the formation of products, such as tea producing and fermentation of red wine, while others need to be avoided, such as the aggregation and browning occurred in fruit juice. Nowadays, the food industry is facing challenges of preserving the highest possible quality of natural food products obtained after processing. On the base of well understanding the changes and applications of oligomeric flavonoids, especially proanthocyanidins, in food processing, better and more efficient actions for retaining these nutriment compounds that are sensitive to temperature or pressure can be obtained.

6.6.1 Tea

Fresh tea leaves are rich in flavonoids, especially flavan-3-ol and its dimer and trimer. In order to process them into different products, tea leaves should go through many processing steps including withering, rolling, fermentation, post-fermentation, drying, roasting, etc. Catechins and proanthocyanidins in tea leaves are sensitive to enzymatic and nonenzymatic oxidation. Processing techniques take advantages of changes in these compounds to create the unique flavor and quality of tea.

Tea polyphenols including catechin, epicatechin, epigallocatechin gallate, and epicatechin gallate mainly exist in the chloroplast and vacuole, while the oxidation-polymerase enzyme mainly exists in the cytoplasm. High intensity of mechanical actions leads to the deformation of tea cells and rupture of the outer membrane of chloroplast, causing catechin substrate to contact and react with oxidase. This process facilitates the formation of catechin polymers in tea cells, such as proanthocyanidin dimers and theaflavins (Chen et al. 2020). Subsequently, polyphenol oxidase activity is passivated rapidly by high temperature to prevent the enzymatic oxidation of tea polyphenols in fixation step.

Preparations of black tea and brick tea both require a fermentation process. By fermentation, the enzymatic browning and oxidation of tea polyphenols give the tea a dark red color. In this period, the oxidation of flavan-3-ols by polyphenols oxidase produces series of benzotropolone compounds, including theaflavin and its gallate derivatives as well as theasinensins. Meanwhile, the catechin monomer contents are decreased correspondingly. The oxidation products of catechins play a decisive role in the color, aroma, and quality of black tea. In addition, theaflavins could only be detected when black tea was heated up to 80 °C, resulting from the degradation of thermosensitive intermediates (Tanaka et al. 2010). In oolong tea, the highest content of total proanthocyanidins was in fresh tea buds before processing, and then decreased by about 20% after fermentation and roughly 15% after drying. During semi-fermentation, some catechins may be oxidized and polymerized to form

theaflavins (Dou et al. 2007). Thearubigins is the deep oxidation products of catechin and tea polyphenols, accounting for 20% of the total solid content of black tea. Besides, the degradation products of theaflavins, namely, epitheaflavic acids, play an important role in the formation of thearubigins. Different from thearubigins, theabrownin is a pigment of brick tea formed in the post-fermentation, and the chemical structure is still unclear. The tea processing technologies make full use of the oxidation, browning, and degradation of biflavonoid and oligomeric flavonoids, and the resulting complex products are further contributed to the color, flavor, and quality of the tea.

6.6.2 Red Wine

Proanthocyanidins, as typical bitter substances in red wine, can effectively stabilize anthocyanins, increase the complexity of wine flavor and affect the clarity and stability of the wine. Generally, wines made from matured grapes contain a higher proportion of proanthocyanidins than that made from immatured grapes, as the content and polymerization degree of proanthocyanidins increase with grapes ripening. In addition, proanthocyanidins are found mainly in grape skins, seeds and marginally in grape flesh, whereas the mean degree of polymerization of proanthocyanidins in seeds is lower than that in flesh. To some extent, molecular size of proanthocyanidin and it's monomer composition, have considerable influences on the perception of convergence. Greater degree of polymerization and percentage of galloylation of proanthocyanidins usually cause more astringent of wine (Gil et al. 2012).

Postharvest dehydration has been shown to significantly improve the leaching and release of proanthocyanidins in the subsequent process. Besides, postharvest dehydration reduces the water content and protects the flavonoid ingredients by slowing down the oxidation rate of these compounds (Segade et al. 2016). Through dehydration, the seeds show a significant increase in proanthocyanidin concentration and a decrease in degree polymerization, while the amount and the mean polymerization degree of proanthocyanidins in skins are both decreased (Moreno et al. 2008). During grape maceration, proanthocyanidins are dissolved from grape skins and seeds. The concentration of proanthocyanidins in wine tends to increase with the extension of maceration time, but the degree of polymerization decreases (Busse-Valverde et al. 2012). The proanthocyanidin content of grapes pretreated with cold maceration is significantly increased after pressing, by promoting the release of proanthocyanidin in grape seeds. The use of maceration enzyme can also further increase the proanthocyanidins released from grape skins and seeds (Busse-Valverde et al. 2012).

The levels of flavan-3-ol, catechin, and epicatechin decline in micro-oxidized wines. Radicals and quinones formed during the oxidation process can be polymerized to form larger proanthocyanidins, reacting with anthocyanins to form new pigments or precipitate. Thus, the decreases of flavanols and oligomeric proanthocyanidins are most likely due to the formation of new pigments (Carrascon

et al. 2015). The concentration of SO_2 may affect the method of forming new tannins and polymerized pigment compounds that proanthocyanidins combine with other polyphenols, including anthocyanins. With a low concentration of SO_2 in wine, the monomer anthocyanins and flavan-3-ol are also significantly reduced, while tannins and non-bleached pigments are increased. When treated with high SO_2, these changes in wine are largely suppressed. During aging, proanthocyanidins react with anthocyanins, causing the decrease of free anthocyanins rapidly and increase of polymerized anthocyanins. Therefore, the color of wine gradually changes from the initial purplish red to brick red. Especially during the aging process in oak barrels, the concentration of proanthocyanidins descends, but the degree of polymerization increased, on account of the formation of some polymer precipitation by the combination of high polymer and micro-oxidation. Therefore, with the increase of proanthocyanidins' polymerization degree and precipitation, the convergence of red wine becomes moderate (del Carmen Llaudy et al. 2006).

To ensure the clarity and bright color of wine properties and stability of shelf life, fermented wine will go through the clarification processes. Conventionally, the application of clarifiers, such as egg whites (Martínez-Lapuente et al. 2017) and plant proteins (Granato et al. 2014) as well as some clarification techniques, like filtration and centrifugation, can improve the wine's clarity. Some flavonoids and polysaccharides in wine will be adsorbed by the membrane materials. In addition, removal of grape seeds results in 40% lower of proanthocyanidin level in wine, compared to that made from whole grapes. This demonstrates the nutritional superiority of fermentation by the whole berry. Other techniques, such as maceration enzymes treatment or cold extraction, preferably enhance the color and improve the content of seeds proanthocyanidins in the wine (Bautista-Ortín et al. 2014). During the whole fermentation process, proanthocyanidins also play a strong antioxidant role in effectively avoiding the oxidation of anthocyanins and leading the favorable change of wine color.

6.6.3 Juice

Many fruits (berry, apple, peach, hawthorn, etc.) with abundant proanthocyanidins are always processed into juice products in order to facilitate transportation and consumption. The processing needs to go through blanching, squeezing, enzymatic hydrolysis, extrusion, concentration, clarification, pasteurization, storage, and other series of steps. These steps can affect the content of proanthocyanidin and its polymerization degree.

Blanching is necessary to protect the color of fruits and inactivate the enzyme activities. It also helps to change the permeability of the cell membrane so that the nutrients can be dissolved out more easily. After blanching, proanthocyanidins are stable in chokeberry juice (Wilkes et al. 2013); oligomeric proanthocyanidins of juice increases in cranberries (White et al. 2011).

The most significant loss of proanthocyanidins is caused by squeezing and the removal of skins and seeds. After squeezing, proanthocyanidins are mostly retained

in pomace, and the extraction amount is between 20% and 40%. Release of proanthocyanidins during crushing could be further improved by preheating or enzymatic hydrolysis of mash (Toydemir et al. 2012). Comparing with several squeezing technologies, the content of procyanidin B2 in juice is the lowest by the rack-and-frame press and is the highest by belt press. However, juice processed by belt press has the worst sensory quality, characterized by less sweetness, more acidity, more bitterness, and more astringent (Heinmaa et al. 2017).

Enzymatic hydrolysis could improve the content of proanthocyanidins in juice. Using pectinase to treat chokeberry in 95 °C for 3 min before the mash press, the concentration of proanthocyanidins is raised by 11% (Wilkes et al. 2013). As for apple juice, the proanthocyanidin content is increased by pectinase treatment. Nevertheless, the treatment is adverse for the stability of cloudy juice, reducing the viscosity of the product by the high hydrolysis of pectin (Oszmiański and Wojdyło 2007).

Some heat treatments, like concentration and extrusion, are not conducive to the retention of proanthocyanidins. Extruded grape seeds and pomace contain 120% and 80% of proanthocyanidins respectively than those in materials without extrusion (Khanal et al. 2009). The concentration of proanthocyanidins in blueberry and grape residue can be also significantly reduced by heating. When heating around 60 °C, the concentration of proanthocyanidins decreases. However, heating for 3 days at a low temperature might be harmless, while heating for more than 8 h at a high temperature resulted in a significant loss of proanthocyanidins (Khanal et al. 2009). For example, the monomers, dimers, trimers, tetramers, pentamers, hexamers and heptamers in peaches were reduced by 11%, 9%, 12%, 6%, 5%, 30% and 30% respectively during the heat treatment. After 3 months of aging, monomer, dimer, trimer, and tetramer levels in canned peach dropped by 10%, 16%, 45%, and 80%, respectively (Hong et al. 2004).

Chitosan and gelatine treatments, used in clarification of apple juices, both can cause the decline of proanthocyanidins content and polymerization (Oszmiański and Wojdyło 2007). A similar phenomenon was observed in blueberry juice treated with 10 $g \cdot L^{-1}$ cyclodextrin that can be used as a protective agent for phenolic compounds, especially proanthocyanidins, in fruit juice (Kelanne et al. 2019). In different blueberry products, there were only 19% and 23% proanthocyanidins retained in unclarified and clarified juices and 41% in dense mixtures. Monomer and dimer retained more than the high polymers in all products after processing (Brownmiller et al. 2009). Conlusively, clarification will result in the reduced content of proanthocyanidins and retain more oligomeric proanthocyanidins in fruit juice.

The proportion of the low molecular weight of polymeric pigments in pasteurized juice is higher than that in aged juice, and the trend of high molecular weight is opposite. Proanthocyanidins with anthocyanins would form polymers, usually referred to as polymeric pigments, during the processing. A larger scale of polymerization will also occur during long-term storage, after which the degree of polymerization of proanthocyanidins was finally determined to range from 1 to 8 in chokeberry (Howard et al. 2012). Pasteurization was proved to have no evident

effect on proanthocyanidins in grape juice (Genova et al. 2016) and cranberry juice (White et al. 2011). In peach pieces, proanthocyanidin B1 changed slightly from 14.7 μg·g^{-1} to 15.8 μg/g after pasteurization (Oliveira et al. 2012), in contrast to highbush blueberries juice (Skrede et al. 2000). Considering the possible relationship with pre-cold extraction, the phenolic compounds like proanthocyanidins of fruit juices are not usually changed by the application of pasteurization and even increased. Sometimes the rise of temperature may promote the release of flavonoid compounds. For example, proanthocyanidins in chokeberry juice were increased by 17% after pasteurization (Wilkes et al. 2013).

The concentration and degree of polymerization of proanthocyanidins will change during the storage period. More monomer and flavan-3-ol dimers were observed after 30 °C storage. The possible reason for such changes can be ascribed to the depolymerization of proanthocyanidins and the conversion to basic units (Wojdyło et al. 2014). Meanwhile, the polymeric proanthocyanidins probably combine with some macromolecules such as polysaccharides and proteins in juice and then form precipitation, which leads to a decrease in the determination of the polymeric proanthocyanidin content. The high polymers found in the sedimentation demonstrate that the higher the polymerization degree of proanthocyanidins, the easier the polymerization occurred. Proanthocyanidin compounds of hawthorn fruit and beverage were stable at 4 °C and relatively unstable at 23 °C and 40 °C. Procyanidin B2 was significantly degraded by 50% and 30%, respectively in fruits and beverages after 6 months of storage at 23 °C, and almost completely degraded after 6 months at 40 °C (Chang et al. 2006). Pre-treatment before storage is beneficial for the quality of fruit products during the storage period. For instance, adding L-ascorbic acid had a protective effect on proanthocyanidins because of the competition for the substrate of the polyphenol oxidase (Kolniak-Ostek et al. 2013).

Although high levels of proanthocyanidins may represent more nutritional and functional benefits, they are not conducive to sensory evaluation, which may be not very acceptable to consumers. Some sensory properties (color, bitterness, and astringency) of juice can be controlled by modulating the squeezing conditions. The pressure, speed, and environment of pressing will affect the content of proanthocyanidins. The rising temperature during pressing results in the transition of bitter or astringent proanthocyanidins, and the paste can be oxidized to reduce bitterness and astringency. Meanwhile, filling with inert gas or isolating air during the period will have a protective effect on proanthocyanidins.

6.6.4 Cocoa

Raw cocoa beans contain procyanidin A2 (9–13 mg/kg) and procyanidin B2 (340–650 mg/kg). After fermentation, procyanidin A2 in cocoa beans is partially degraded and epimerized, whereas a second epimer emerges after roasting (De Taeye et al. 2017). The stability of monomer and dimer proanthocyanidins in cocoa beans is dependent on pH value. When pH $>$ 6, epicatechin, catechin, procyanidin B2, and procyanidin B5 become unstable and then degrade almost completely within a few

hours. Whereas at pH 5.0, they are relatively stable. Additionally, ascorbic acid has been found to promote the stability of monomer and dimer of proanthocyanidins in cocoa, which is not dependent on the decrease in pH but as an antioxidant agent (Zhu et al. 2003).

6.6.5 Other Food Productions

Proanthocyanidins are found to exist in cinnamon. When cinnamon is peeled from the branches, the surface of cinnamon wood immediately changes from white to reddish-brown, due to the reaction of proanthocyanidins with aldehydes catalyzed by enzymes (Tanaka et al. 2010). *Coffea arabica* leaves, a substitute for the traditional tea, contain procyanidins B and C as well. Being dried at different temperatures, the content of procyanidin B in these leaves increases with the rising temperature and reaches the highest content at 50 °C (57.79 mg/g). Additionally, young leaves have higher level of proanthocyanidins than older leaves, indicating that the content of proanthocyanidins in leaves is closely related to the maturity of leaves (Ngamsuk et al. 2019). Some biflavonoids abundant in *Semecarpus anacardium*, *G. cambogia*, and *G. gingko* has not been reported relevant changes in the application of food. In a word, the changes of biflavonoid and oligomeric flavonoid, represented by proanthocyanidins, in food processing and application are complex and attractive.

6.7 Safety: Toxicity and Side Effects

There are few studies on the toxicity and side effects linked with proanthocyanidins. The prevalent view is that the procyanidins are beneficial natural plant constituents with low risk of side effects or drug interactions. They have an excellent safety profile, without obvious side effects, toxicity, either drug interactions. Human safety and toxicity studies for proanthocyanidins are limited.

Side effects of proanthocyanidins are rare, but when they do occur, they are limited to occasional allergic reactions and mild digestive distress (Schulz et al. 2001). One study unexpectedly found that a combination of proanthocyanidins and vitamin C might slightly increase blood pressure in people with high blood pressure. Neither treatment alone had this effect (Ward et al. 2005). These results may become a statistical fluke, but nonetheless people with hypertension should be careful when they simultaneously consume vitamin C and proanthocyanidins. One study, though, has found that Pycnogenol (pine bark extract) may help improve kidney function in people with metabolic syndrome who take high blood pressure medicine (Stuard et al. 2010). The maximum safe dosages for young children, pregnant or nursing women, or those with severe liver or kidney diseases have not been established. Additionally, proanthocyanidins may have some anticoagulant properties when taken in high doses and therefore should be used only under medical supervision by individuals on blood-thinner drugs, such as warfarin

(Coumadin), heparin, clopidogrel (Plavix), ticlopidine (Ticlid), pentoxifylline (Trental), or aspirin. Meanwhile, some interactions we should know include high doses of proanthocyanidins that might cause a risk of excessive bleeding, if you are taking warfarin (Coumadin), heparin, clopidogrel (Plavix), ticlopidine (Ticlid), pentoxifylline (Trental), or aspirin.

The supplement with compounds like pine bark extract that contains proanthocyanidins is believed to be safe in almost all cases. However, there are few groups who should not supplement with procyanidins. They include gestating or nursing women, people with bleeding disorders, anyone scheduled for surgery in the near future, individuals with systemic diseases like lupus and multiple sclerosis, and others according to their doctors' recommendations. Thus, in order to be sure that you are not at risk for any possible side effects of procyanidin supplementation, it is best to talk about it first with the personal doctor.

6.8 Marketed Products

The pursuit of health and long life is the eternal wish of the people, which brings a huge consumption of natural products with health-promoting effects. Based on the profits from the researches and propagation of firms, many biflavonoid and oligomeric flavonoid products are widely accepted by consumers. However, due to the high production costs, pure biflavonoids and oligomeric flavonoids are rarely used in commercial products, whereas the products with guaranteed healthy functions and content of bioactive components are mostly popular in the market.

Many extracts with biflavonoids and oligomeric flavonoids from various plants are marketed openly, and oral products are the most familiar form. For instance, apple, berry, grape seed, tea, and cinnamon are used to make the proanthocyanidin-rich health products. It makes consumer intake the functional molecules without special eating of these foods. Furthermore, a kind of pine barks extracts with a commercial name of Pycnogenol, which is not from foods but can also be used as an oral supplement. Meanwhile, the products are carefully designed for different target people, such as children, elderly people, and middle-aged persons who are classified and sold separately. There are also some proanthocyanidin extracts, especially from apple, used as cosmetics and conditioners.

In addition to the proanthocyanidins, products which contain biflavonoids are also a class of health supplements with wide acceptance. The extracts from *G. Cambogia* and *G. kola* can be easily purchased on the market as a kind of product for losing weight. For example, a beverage made from the leaves of *G. biloba* has been used as traditional Chinese medicine. *G. biloba* nuts (also called Baiguo), also a kind of vegetable in food, are used as a popular snack in China and Japan. In addition to direct consumption, the powder and extract of the leaves and the extracts of the fruits are also a health supplement on the market.

Essentially, the good-selling products with biflavonoids and oligomeric flavonoids, due to the health promotion of these molecules, can partly meet the health

demand of contemporary people. It can be expected that there may be more new biflavonoid and oligomeric flavonoid products on the market in the near future.

6.9 Patents

In recent years, many patents on proanthocyanidin and biflavonoids have been published, ranging from the preparation, purification, and modification methods and the identification of biological functions to their applications in food, medicine, cosmetics, and other industries. Some patents have described the extraction of proanthocyanidin and biflavonoids from flavonoid-rich plants, such as *Garcinia*, palm infructescence, black wattle bark, *Selaginella tamariscina*, *Daphniphyllum oldhami*, *Stellera chamaejasme* vegetal, Chinese arborvitae twig and leaf, ginkgo leaves, and so on. Some patents have proved many biological functions of proanthocyanidin and biflavonoids, e.g., scavenging free radicals, improving sleep, antivirus, antitumor, anticancer, antiallergic, and anti-inflammatory activities. Proanthocyanidin and biflavonoids are widely used in the pharmaceutical industry, and some compositions have been proved helpful to many disease and disorders, including gastric ulcer, a mineralocorticoid receptor-related disease, inflammation, diarrhea, oropharyngeal bacterial colonization, glucose and lipid metabolism disorder, atherosclerosis, asthma, gout, renal lithiasis, acne, frostbite, obesity, hypercholesterolemia, and so on. Several patent applications have proposed some novel methods for the preparation of proanthocyanidin and biflavonoids with known biological activities. Proanthocyanidin and biflavonoids are also used in the food industry, such as drinks, teas, wines, yoghourt, functional foods, healthcare foods, and food supplement.

Table 2 has listed the relevant international patents published by various countries over last decades about proanthocyanidin and biflavonoids, and these patents are classified by the contents (preparation, purification, modification, or application of active compositions).

6.10 Perspectives and Recommendations

The concern and enthusiasm for biflavonoids and oligomeric flavonoids from food have never decreased in the past decades, either in research areas or public health. However, proanthocyanidins are the most focused one, and other compounds were overlooked. In fact, there are many ancient traditional foods and new healthy food with unexplored biflavonoids and oligomeric flavonoids into the modern human life step by step. The bioactivity, function, benefits, and potential health risk of these molecules are all required to be carefully investigated. Some products that contain flavonoid polymers with uncertain activity and toxicity have already been promoted to the market. The public health benefits and unknown risks of these foods or supplements all need continued attention. Apart from the benefits of biflavonoids and oligomeric flavonoids, the bitterness, astringency, and intestinal stimulation

Table 2 International patents published in the last 20 years about proanthocyanidin and biflavonoids, based on preparation, purification, modification, and application of active compositions. Source: https://worldwide.espacenet.com. Last accessed: Nov 29, 2019

Oligomeric flavonoids		Patent name	Application number
Proanthocyanidin	Preparation, purification, and modification	Preparation method and applications of palm infructescence proanthocyanidins	CN201910132127A20190222
		Method for producing low polymerization degree proanthocyanidin and proanthocyanidin having low polymerization degree produced by said method	JP2012076002W 20121005
		Separative purification method of proanthocyanidin	JP2009113527A 20090508
		Method for extracting proanthocyanidin from peanut coats	CN201110450591A20111229
		Method of producing proanthocyanidin oligomer	AU2012244090A 20121019
		Black wattle bark proanthocyanidin microcapsule and preparation method thereof	CN201310208058A20130530
		Method for purifying oligomeric proanthocyanidin, method for controlling degree of polymerization, hyaluronidase inhibitor, and collagenase inhibitor	CN201280062680A20121227
		Method of producing proanthocyanidin oligomer	US201514976645A20151221
		Preparation method for quickly separating plant proanthocyanidin dimer and trimer	CN201610946727A20161026
		Preparing method for uniform-component	CN201711382001A20171220

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		oligomeric proanthocyanidin	
		Process for producing a proanthocyanidin extract	CA2692688A 20080707
		Methods for purifying proanthocyanidin oligomers	EP00906694A 20000303
		Preparation method and application of proanthocyanidin derivative	CN201310142881A20130424
	Application of active compositions	Sea buckthorn proanthocyanidin soft capsule for treating gastric ulcer by killing helicobacter pylori and preparation method thereof	CN201711333742A20171214
		Cosmetic use of proanthocyanidin A2	US201013142661A20100127
		New use of proanthocyanidin B2	CN201310636601A20120620
		Use of a-type proanthocyanidins in treating a mineralocorticoid receptor related disease	EP13178151A 20130726
		Method of treating inflammatory diseases and medicine application of using proanthocyanidin compound to treat inflammatory diseases	CN201610465902A20160623
Oligomeric flavonoids		**Patent name**	**Application number**
Proanthocyanidin	Application of active compositions	Methods of treating diarrhea in adult nonhuman animals	US201515314171A20150528
		Application of proanthocyanidin compounds to preparation of drug against anti-Zika virus	CN201710703223A20170816
		Oligomeric proanthocyanidin extract mixed STOCK solution capable of	AU2019100706A 20190627

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		scavenging free radicals from air and preparation and use methods thereof	
		Grape seed proanthocyanidin additive and preparation method and application thereof	CN201610195983A20160331
		A proanthocyanidin natural healthcare beverage and preparation method thereof	CN201510203065A20150424
		Use of cranberry proanthocyanidin for treatment of oropharyngeal bacterial colonization	AU2015246049A 20150409
		Use of croton- or calophyllum-derived proanthocyanidin polymers or botanical extracts in combination with rifaximin for the treatment of diarrhea in nonhuman animals	US2016019337W 20160224
		Application of proanthocyanidin compound to preparing products for preventing and/or treating glucose and lipid metabolism disorder	CN201811233691A20181022
		Phospholipid complexes of proanthocyanidin A2 as antiatherosclerotic agents	US85780401A 20010611
Biflavonoids	Preparation, purification, and modification	Biflavonoids compounds and preparation method and pharmaceutical applications thereof	CN201510736739A20151103
		Preparation and new usage of *Selaginella tamariscina* biflavone ingredient	CN200910066695A20090326
		Synthesis of C-3 coupled biflavonoids	KR20127004402A 20100819

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		and C-3 coupled biflavonoid analogs	
		Method for separating and extracting two types of biflavone from *Daphniphyllum oldhami*	CN200910155221A20091208
		Biflavonoid compound and application thereof	CN201810648374A20180622
		Biflavonoid compound as well as preparation method and application thereof	CN201810098902A20180131
		Biflavonoid-cobalt complex, preparation method for same, and applications thereof	CN2018076720W 20180213
		Biflavonoid-iron complex, preparation method therefor, and use thereof	CN2018076732W 20180213
		Agricultural bacteriostat of *Stellera chamaejasme* vegetal biflavonoids and preparation method thereof	CN201010000597A20100114
		Method for extracting *Selaginella biflavone*	CN201110117997A20110506
		Method for preparing biflavone capsules and tablets	CN201110096867A20110414
Oligomeric flavonoids		**Patent name**	**Application number**
Biflavonoids	Preparation, purification, and modification	Method for extracting biflavone from Chinese arborvitae twig and leaf	CN201810072711A20180125
		Method for extracting biflavone compound from ginkgo leaves	CN201811146323A20180926
		Biflavone-zinc complex, preparation method therefor, and use thereof	CN2018076739W·2018-02-13
		Biflavone-nickel complex and preparation method and application thereof	CN201710097723A20170222
		Biflavone-copper complex and preparation	CN201710097599A20170222

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		method and application thereof	
		Biflavone-manganese complex, as well as preparation method and application thereof	CN201710097622A20170222
		Ginkgo extract with low content of 4′-O-methylpyridoxine and/or biflavone	JP2009063133A 20090316
		Method for preparing podophyllotoxin, biflavone of rhizoma et radix *Dysosma pleiantha* and analogs thereof	CN201710039609A20170119
		Application and preparation method of *Garcinia xanthochymus* leaf extract	CN201610570472A20160719
		Process for preparing extract containing hypericin and flavon compounds	CN02134379A 20020718
		Garcinia buchananii baker compounds, compositions, and related methods	US2012071441W 20121221
		Preparation method of theaflavin	CN201410667750A20141110
		Theaflavin preparation device	CN201720729740U20170622
		Theaflavin fermentation tank	CN201220642599U20121129
		Methods of making and using theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin 3,3′-digallate and mixtures thereof	US23516308A 20080922
		Preparation method of theaflavin composite	CN201710099454A20170223
		Catalyst for synthesizing theaflavin and method	EP15811544A 20150629

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		for synthesizing theaflavin	
		Method of producing theaflavin and beverage comprising theaflavin	JP2013269169A 20131226
		Industrial preparation method of theaflavin	CN200910153520A20090930
		Theaflavin extraction and purification method	CN201010539524A20101111
		Preparation method of theaflavin monomer	CN201110156318A20110610
		Method for extracting theaflavin from black tea	CN201610574681A20160719
		Spray drying system for theaflavin production.	CN201220642596U20121129
		Preparation method of theaflavin microemulsion	CN201610197665A20160331
		Method for preparing high-purity theaflavin composite	CN201210007418A20120112
		Method for producing theasinensin	JP2008315488A 20081211
		Tea making technology for increasing theaflavin content	CN201710708498A20170817
		Bottled theaflavin-containing food and drink and method for stabilizing theaflavin-containing food and drink	JP2017069686A 20170331
Oligomeric flavonoids		**Patent name**	**Application number**
Biflavonoids	Preparation, purification, and modification	Method for synthesizing theaflavin by immobilized enzyme method	CN200910154635A20091123
		Production technology of high theaflavin instant black tea	CN201610500144A20160628
		Preparation method for tea leaves with high theaflavin content	CN201410552637A20141017
		Processing technique of high-aroma high-	CN201811024639A20180904

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		theaflavin summer black tea	
		Novel feed mixing equipment with theaflavin extract product adds device	CN201820764503U20180522
		A method for preparing four kinds of theaflavin monomer	CN200610154852A20061124
	Application of active compositions	Application of ginkgo biflavonoids to preparation of medicine for preventing and treating asthma	CN201711146147A20171117
		Application of sciadopitysin in preparation of medicines for prevention and treatment of diabetes	CN201310032630A20130128
		Anti-influenza virus compound comprising biflavonoid-sialic acid glycoside	EP03703370A 20030225
		Anti-wrinkle composition for external applications to the skin containing biflavonoid derivative	KR20070053700A 20070601
		Composition comprising biflavonoid derivative showing anti-inflammatory and antiallergy effect	KR20060055964A 20060621
		Application of ginkgo biflavonoid compound in preparation of slimming medicines and/or sliming medicine compositions	CN201810832435A20180726
		A composition comprising bioflavonoid derivatives or salts thereof for preventing, improving, or treating metabolic disease caused by lipid unbalance	KR20160163293A 20161202
		Use of oxygenated heterocycles chosen	EP2008057808W 20080619

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		from xanthones and biflavonoids for the preparation of a composition intended to act as an anti-coccidial agent	
		Pharmaceutical preparation of biflavone compound for anti-gout	CN200710052157A20070514
		Application of fenugreek biflavone glycosides for preparing antivirus or/and antitumor drugs	CN201010533597A20101106
		Biflavone compound and uses thereof for treating cancers and preparing drugs	CN2016080931W 20160504
		Application of three biflavone monomer components extracted from ginkgo leaves in preparing medicament of alpha-glucosidase inhibitor	CN200910067430A20090824
		Novel use of natural amentoflavone in curing viral diseases	CN200910248591A20091221
		Anti-glycation agent comprising a *Garcinia kola* extract or fraction	US201314083768A20131119
		Skin care preparation for bleaching use	JP2003278417A 20030723
		Pharmaceutical composition for curing DN (diabetic nephropathy) and preparation method of pharmaceutical composition	CN201510871910A20151203

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
Oligomeric flavonoids		**Patent name**	**Application number**
Biflavonoids	Application of active compositions	A method of treatment for acne and an anti-acne formulation	CN201380013647A20130312
		Traditional Chinese medicine preparation capable of clearing stomach and discharging fire and preparation method of traditional Chinese medicine preparation	CN201410631122A20141112
		Traditional Chinese medicine preparation for moistening dryness and relaxing bowel and preparation method thereof	CN201410631096A20141112
		Ointment preparation for treating frostbite and preparation method thereof	CN201610009204A20160108
		Taxus chinensis fruit healthcare ferment and preparation method thereof	CN201510346132A20150619
		Dietary supplement derived from natural products by hot melt extrusion (hme) processing	IB2018051998W 20180323
		Theaflavin tablet and drink	CN200610026525A20060512
		Method of using theaflavin	US201414527610A20141029
		Theaflavin-containing feed additive	CN201810989269A20180828
		Packaged drink containing theaflavin	JP2008267472A 20081016
		High concentration theaflavin-containing drink	JP2008268219A 20081017
		Application of theaflavin and skin care product containing theaflavin	CN201310025119A20130123
		Application of theaflavin in pharmacy	CN200910094073A20090202

(continued)

Table 2 (continued)

Oligomeric flavonoids		Patent name	Application number
		Leptin secretion promoter using theaflavin	JP2017033553A 20170224
		Theaflavin yoghourt and preparation method thereof	CN201811301458A20181102
		Application of theaflavin-3, 3′-digallate	CN201610591899A20160726
		Neutral fat-reducing agent and suppressor of body fat increase	JP2008067688A 20080317
		Cholesterol-lowering agent	JP2009035709A 20090218
		Theaflavin matt glaze and manufacturing method thereof	CN201210441475A20121108
		Preparation method of rich theaflavin black tea	CN201110222334A20110804
		Method for synchronously preparing high-theaflavin broken black tea and high-theaflavin instant black tea powder	CN201410561627A20141021
		Packed drink containing theaflavin compound and method for controlling decrease of theaflavin compound content in drink	JP2008265324A 20081014
		Tea leaf pre-treatment device used in theaflavin production process	CN201320293864U20130527
		Medical application of theaflavin-digallate to preparation of MBLs inhibitor	CN201810802295A20180720
		Application of theaflavin as synergist of antifungal medicine	CN201010157469A20100427
		Application of theaflavin to preparation of tyrosinase inhibitor	CN201210314600A20120830

toward people ranging from all ages should be considered. The taste preference of people varies with ages, which may affect the total intake amount of these compounds from diets.

Although there are considerable studies on the *in vivo* metabolism and bioavailability of biflavonoids and oligomeric flavonoids, with hypothesizing that these compounds are frequent intake from daily diets, it is believed that the research should be further expanded, especially in terms of the relationship between metabolic processes and health promotion. In addition, the low bioavailability of these phytochemicals does not mean low biological activity *in vivo*. Interaction between flavonoid polymers and gut microbiota is one possible way to achieve active functions *in vivo*. Thus, the effect of these molecules, especially oligomeric flavonoids, on the gut microbiota needs to be clarified. Besides, the investigation on hypoglycemic, hypolipidemic, anti-inflammatory activity should also be continued, especially *in vivo* researches.

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Soy Isoflavones

7

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Contents

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Abstract

Soy and its bioactive compounds, isoflavones, have become a hot issue in the last 20 years. Increased breeding of the plant has created a strong market in many countries. Nevertheless, the cross-consumption of foreign products is not safe for certain ethnic groups and requires risk assessment evaluation because of ethnic differences among populations that induce, for certain compounds, different end points in humans. For example, in Asians and white people, the intake of these compounds can have different bioactive effects. Chemically, isoflavones are isoflavonoids, a subgroup of polyphenolic compounds that have affinity for estrogen receptors and can act as endocrine disruptors in white people. Isoflavones from soy can also be found as constituents of astragalus roots, adzuki beans, chaste, green peas, chickpeas, lupins, kudzu, and red clover, among other plants. An extract named okara also contains isoflavones in variable concentrations and is a by-product of the production of soy preparations, such as tofu and soybean beverages. The main isoflavones isolated from soy are the malonyl glycosides of genistein, daidzein, and glycitein; these aglycones appear only in very low levels. All of these compounds undergo enterohepatic metabolism and are detoxicated by cytochrome P450 (CYP) 1A2 and 3A4, which imply an important potential to induce drug–herb interactions with concomitant intake. Recent research on bioactivity/toxicity of isoflavones has provided a perspective for potential health improvement, but sometimes these results are hard to compare in different ethnic groups. Correct evaluation of each extract in animal and clinical trials is the key to understanding the end points in order to assess safety. This chapter discusses, in detail, the future challenges for isoflavone-derived products, especially in the Western market.

Keywords

Soy · Isoflavone · Genistein · Daidzin · Ethnic · Estrogen-like · Disruptor · Hormone · Cancer · Tumor · Proliferation · CYP 3A4

Abbreviations

λ_{max}	Maximum wavelength
ANF	Antinutritional factor
CID	Compound ID
COMT	Catechol-*O*-methyltransferase
CVD	Cardiovascular disease
CYP	Cytochrome P450
DAD	Diode array detection
DBP	Diastolic blood pressure
EFSA	European Food Safety Authority
E_{max}	Maximum effect
ER	Estrogen receptor
ESCO	EFSA Scientific Cooperation

ESPGRAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
FLS	Fatty liver syndrome
FSH	Follicle-stimulating hormone
GABA	γ-Aminobutyric acid
GHO	Global Health Observatory
GnRH	Gonadotropin-releasing hormone
HDT	Hormone-dependent tumor
HPLC	High-performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
MeSH	Medical Subject Heading
MF	Molecular formula
MRS	Menopause Rating Scale
MW	Molecular weight
NCCAM	National Center for Complementary and Alternative Medicine
NCCIH	National Center for Complementary and Integrative Health
NPGS	National Plant Germplasm System
OTC	Over-the-counter
RT	Retention time
SF	Soy-based formula
SBP	Systolic blood pressure
SEER	Surveillance, Epidemiology, and End Results
sh	Shoulder
TE	Theoretical efficacy
TERE	Theoretical efficacy relative to estradiol
TSH	Thyroid-stimulating hormone
UNICAMP	University of Campinas
UNIFESP	Federal University of São Paulo
USDA	US Department of Agriculture
WHO	World Health Organization

7.1 Introduction (Sources and History)

Historically, the soybean (*Glycine max* (L.) Merr.) originally came from China as a basic food ingredient in traditional Asian cuisine, which has been used for thousands of years. The data show that probably the soybean started to be used around 5000 BC (Lee et al. 2011), but the history of the soybean has been informed mainly by historical documents indicating that the soybean was domesticated in East Asia in association with the Zhou dynasty (approximately 2500 years ago) in China. Since 2003, archeological research has brought to light more broad information suggesting that North China was, indeed, the region where the soybean was domesticated. Soybeans have been found at many archeological sites in China, Korea, and Japan. However, the details are still poorly understood. At the beginning of the Mumun period (around 3500 years ago), intensive agriculture was established –

including adzuki beans, soybeans, rice, wheat (*Triticum* cf. *aestivum*), and barley (*Hordeum vulgare*) (Zhao 2010) – and this has continued until the present era. Evidence of the cultivation of crops such as foxtail millet, broomcorn millet, canola (rapeseed (*Brassica rapa*)), and possibly hemp (*Cannabis sativa*) has also been found in the Middle Neolithic Dawenkou culture, which existed in Shandong province in around 4300–2600 BC (Lee et al. 2007). The cultivation of most of these crops appears to have been maintained over the centuries until now.

Soybeans have continued to be consumed over the centuries, but from the outset the populations of these regions learned how to prepare soybean products to avoid the toxicity of isoflavones. The processing of soybeans includes various steps that help to clean the crude material from them. Traditional foodstuffs were traditionally prepared after prolonged simmering, several rinses, and cooking steps in water. These procedures remove isoflavones from the soybean foodstuff. This indicates that the historical exposure to isoflavones was probably low in Asia (Barnes 2010). However, some amounts of these compounds remain in the product – for example, in protein extracts, where there is still a small amount of isoflavones (Fernandez-Lopez et al. 2016). A database of isoflavone content in unprocessed and processed soy products is available from the US Department of Agriculture (USDA) (Bhagwat and Haytowitz 2015).

Soybeans did not reach the Americas and Europe until the eighteenth century; the earliest records of their presence there date back to 1765 in Savannah (Georgia, USA), 1739 in Paris, and 1790 in London (at Kew Gardens). It is only in the last century that the human intake of soy and soy-derived products has increased in these Western countries, with industrialized processing that started in the 1940s (Barnes 2010, with references; Fernandez-Lopez et al. 2016). Nowadays, these products are mainly used for surrogate food production as a protein source solution for vegetarians because of their high protein content and usefulness in the production of different products such as meat analogues. Despite that, doubts remain regarding the effects of these foods in ethnic groups other than Asians and "the potential effects on health, such as the effectiveness on cardiovascular risk reduction or, conversely, on the possible disruption of thyroid function and sexual hormones" (Rizzo and Baroni 2018). This is mainly because isoflavones have a molecular structure that mimics endogenous estrogens, raising doubts about the safety of their use because they can act as estrogen disruptors. The difficulty in the evaluation of their bioactivity is also due to the fact that they are selective estrogen receptor (ER) modulators (Messina 2014). Nowadays, there is more and more information indicating that they are the most prevalent and potent xenoestrogens present in human food (Omoruyi et al. 2013).

This chapter describes a critical multidisciplinary review conducted mostly on the last 10 years of publications, including identification of key literature in this field. Between 2009 and 2013, the author of this chapter contributed, as an expert, to a European Food Safety Authority (EFSA) Scientific Cooperation (ESCO) report titled *Hazard Identification of the Use of Dietary Isoflavones and Isolated Isoflavones from Soy or Red Clover in Food and Food Supplements*, a short public

version of which was made available in 2012. This item was analyzed as a gray literature search, but the confidentiality of the data was maintained. This chapter discusses the knowledge and research developed in this scientific area, including findings from recent reviews in the field, and provides a critical evaluation of the state of the art.

A nonsystematic literature search of Medline and Embase (http://www.ncbi.nlm.nih.gov/pubmed; http://www.embase.com) was performed using the following subject headings/keywords or Medical Subject Heading (MeSH) terms where available. The search was systematized through the following three sets. The first set (a) included soy and soy products; the second set (b) focused mainly on constituents of soy from the phytochemical point of view and nutritional issues; and the third set (c) retrieved information about bioactivity and health purposes in animals and humans:

(a) "Soy" and "soy foods," "soybeans" or "soy beans" or "*Glycine max*" or "soy products"
(b) "Soy protein" or "soy milk" or "traditional soy foods"; "vegetarian" and "soy"; "protein quality" and "isoflavones"; "soy" and "phytochemicals" and "nutrients" or "bioactive compounds"
(c) "Soy" and "isoflavones" and "animal"; "soy" and "cancer" or "soy" and "thyroid" or "soy" and "sex hormones" or "health benefits" or "chronic diseases"

The search was extended to the references within.

The data were summarized, and animal and human information is given here to facilitate critical assessment of the published literature. Part of the hazard identification focuses on the market for isoflavone-rich products, including soy infant formulas, and the data collected should be analyzed in the light of ethnic differences. In this review, we discuss worldwide consumption of soy isoflavones in terms of nutrient composition and bioactive compounds in soy and soy foods. Furthermore, we discuss current evidence of their possible effects on human health and safety. These arguments are relevant to Western countries with growing soy consumption, especially by vegetarians.

7.2 Bioactive Constituents

Isoflavones are polyphenolic compounds from a subfamily named flavonoids. In the majority of flavonoids, the chemical structure includes a phenyl group on the benzopyran ring in position 2, but in the isoflavonoids, this group is in C3 relative to the oxygen in the ring (see Fig. 1). These compounds are also included in the category of phytoestrogens because they mimic the estrogen molecule and have affinity for estrogen receptors (Bennetau-Pelissero 2013), as is explained later in this chapter.

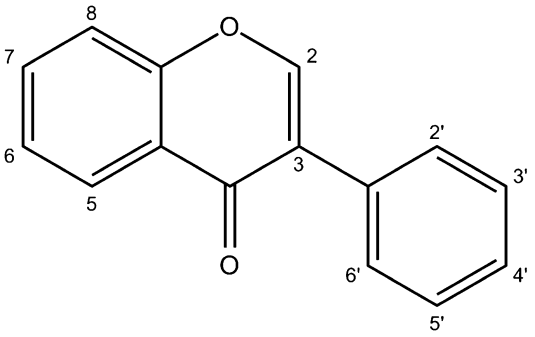

Fig. 1 Basic isoflavone structure. PubChem compound ID (CID): 72304 (3-phenyl-4H-chromen-4-one) (from https://pubchem.ncbi.nlm.nih.gov/compound/Isoflavone)

Chemically, the isoflavone nucleus is a 3-phenylchromen-4-one according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature; in other systems of nomenclature it is known as 3-phenyl-4H-chromen-4-one, 3-phenyl-4H-1-benzopyran-4-one, or 4H-1-benzopyran-4-one, 3-phenyl-, among other names. The molecular formula (MF) is $C_{15}H_{10}O_2$ and the molecular weight (MW) is 222.243 g/mol.

Seeds from the Leguminosae (Fabaceae) species *Glycine max* (L.) Merr., with the common name of "soybeans," are included in a list of species of possible concern because of their content of compounds such as agglutinin (an *N*-acetylgalactosamine-specific lectin), a proteinase inhibitor, and other toxic proteins (BFR 2007; Patisaul 2017).

A similar concern pertains to isoflavones, of which soybeans have an average content of 1–2 mg/g (Campos et al. 2006; http://www.drugbank.ca/drugs/DB12007).

The structures found are mostly malonyl-glycosylated derivatives of genistein (IUPAC name: 5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one; MF: $C_{15}H_{10}O_5$; MW: 270.24 g/mol), daidzein (IUPAC name: 7-hydroxy-3-(4-hydroxyphenyl) chromen-4-one; MF: $C_{15}H_{10}O_4$; MW: 254.241 g/mol), and glycitein (IUPAC name: 7-hydroxy-3-(4-hydroxyphenyl)-6-methoxychromen-4-one; MF: $C_{16}H_{12}O_5$; MW: 284.267 g/mol), which account for an average of 70–50%, 40–20%, and less than 10% of the isoflavones, respectively (Campos et al. 2006). The total isoflavones in soy can also be expressed as glycosides and malonyl glycosides of the main aglycones – for instance, 67–516 μg/g daidzin, 91–1079 μg/g genistin, 12–177 μg/g glycitin, 217–768 μg/g malonyldaidzin, 43–158 μg/g malonylglycitin, 64–2446 malonylgenistin, and 4.3–265 μg/g genistein (http://www.drugbank.ca/drugs/DB12007).

The isoflavone genistein is one of the most active and studied isoflavones and was originally isolated by Perkin and Newbury in 1899 from dyer's broom (*Genista tinctoria*) (Perkin and Newbury 1899). It is the isoflavone most widely represented among many cultivars of *Glycine max*, as discussed previously, and recent studies on it have been mainly related to soy intake.

The extracts found in dietary supplements and in many subproducts in food or supplements have the main isoflavones, conjugated to sugars, cited above; they exist

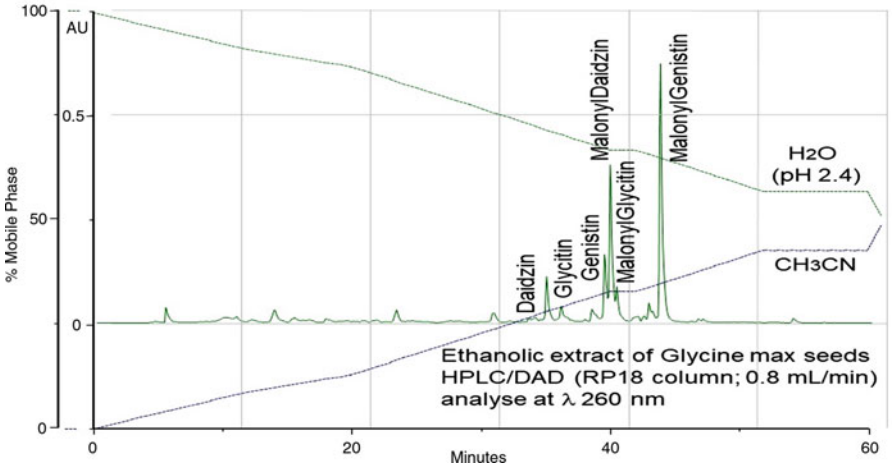

Fig. 2 Relative concentrations of the main isoflavones in an extract of *Glycine max* seeds analyzed by high-performance liquid chromatography with diode array detection (HPLC/DAD) using an RP18 column at 25 °C; the eluent mixture was water (acidified to pH 2.4 ± 0.1 with *o*-phosphoric acid)–acetonitrile gradient with a flow rate of 0.8 ml/min (full information is available in Campos et al. 2007). The chromatogram was acquired at λ 260 nm. Daidzin retention time (RT) 34.8 min, glycitin RT 36 min, genistin RT 39.3 min, malonyldaidzin RT 39.7 min, malonylglycitin RT 40.2 min, and malonylgenistin RT 43.6 min. The sample seeds were graciously provided by the seed bank at the Botanical Garden, University of Coimbra, Portugal

as both simple and complex β-D-glycosides, and in different proportions, which result in a diversity of potential estrogenic effects (Moreno-Franco et al. 2011; Campos and Costa 2012). These are discussed below.

Using high-performance liquid chromatography with diode array detection (HPLC/DAD), analysis of hydroalcoholic extracts is possible to see the main isoflavones in different seed cultivars – for instance, the chromatograms shown in Fig. 2. The most concentrated isoflavones found are the malonyl forms of daidzein, glycitein, and genistein. The last one is the major compound in the extract as malonyl glucoside genistein. Glycosidic forms are also present, as shown in Fig. 2, and are the first shown on the left side of the chromatogram because they have more polar structures.

As shown in Fig. 3, it is possible to analyze the variation in the concentrations of the main isoflavones in extracts of seeds from cultivars of *Glycine max* with similar profiles, using HPLC/DAD with an RP18 column at 25 °C. These samples were prepared with the same relationship between the weight of the sample and the solvent. The HPLC/DAD profiles of these compounds are very similar; however, the concentration of isoflavones is different, with the cultivar Kuro Maru Daizu representing a more concentrated example. More data can be consulted in Campos et al. (2007).

The respective ultraviolet spectra of the various isoflavones from soy, identified in the chromatograms, are shown in Fig. 4.

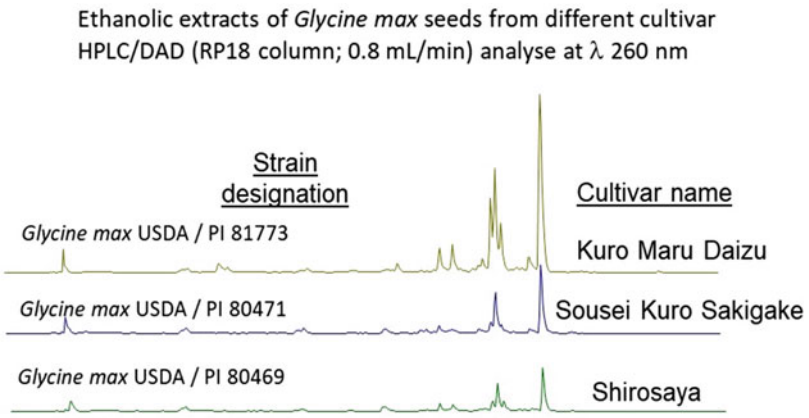

Fig. 3 Relative concentrations of the main isoflavones in extracts of seeds from some cultivars of *Glycine max* with the same profile, analyzed by high-performance liquid chromatography with diode array detection (HPLC/DAD) using an RP18 column at 25 °C. The samples were prepared with the same relationship between the weights of the sample and the solvent. The samples were generously provided by the Germplasm Bank at the US Department of Agriculture (USDA) (freely distributed by the US National Plant Germplasm System (NPGS) for educational, agricultural research, or breeding purposes). None of the seeds used in this study had any form of intellectual property rights protection (data published for the first time). Full information on the samples is provided in Table 2 of Campos et al. (2007)

7.3 Bioavailability and Metabolism (Bioactivity, Nutritional Values, and Functional Aspects)

Following the ingestion of soy foods/beverages/extracts rich in isoflavones, these compounds undergo enterohepatic metabolism. First, they are absorbed in the intestine and further metabolized mainly as glucuronide forms in the liver, then they enter the blood transport system, mainly linked to glycoproteins. Briefly, following ingestion, isoflavone glycosides or their respective aglycones undergo conjugation and give rise to the corresponding metabolites in the plasma once the glycosidic moiety has been hydrolyzed (Setchell et al. 2009; Chandrasekharan and Aglin 2013). The main metabolites are 7-*O*- and 4′-*O*-glucuronides, with small quantities of sulfate esters. A longer transit time for consumed isoflavones glycosides in the large intestine may increase bacterial metabolism of them, which can be crucial, for instance, for the production of certain metabolites such as equol (Zubik and Meydani 2003; EFSA 2012; Liu et al. 2016).

The importance of the microflora in the metabolism of isoflavones was first observed in the 1980s, when studies on germ-free animals showed that formation of equol was dependent on the gut microflora and that antibiotic administration obliterated the conversion into equol (Setchell and Cole 2006; Zhou et al. 2008).

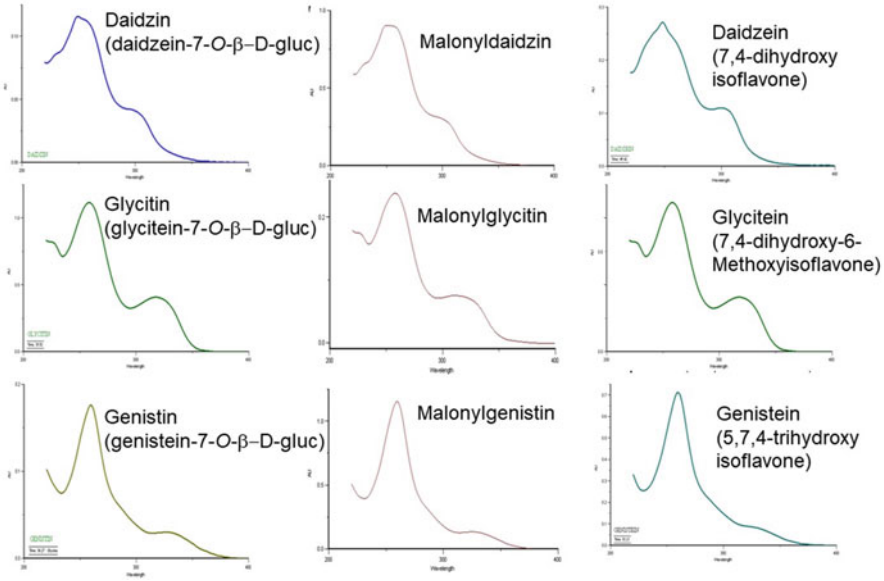

Fig. 4 Soy isoflavones (glycosides, malonyl glycosides, and their respective aglycones). The spectral data were collected between 220 and 400 nm (the spectral zone of the phenolic acids and flavonoids in this study). Daidzin λ_{max} 248.7, 259 (sh), 302 (sh) nm; glycitin λ_{max} 258.7, 320 (sh) nm; genistin λ_{max} 259.7, 328.4 (sh) nm; malonyldaidzin λ_{max} 249–258, 302 (sh) nm; malonylglycitin λ_{max} 258.3, 313.8 (sh) nm; and malonylgenistin λ_{max} 259.4, 330 (sh) nm. λ_{max} maximum wavelength, *sh* shoulder

S-(−)equol (7-hydroxy-3(4′-hydroxyphenyl)chroman) is a metabolite of the soy isoflavone daidzein (the aglycone of the glycoside daizin), which has higher bioavailability and greater affinity for estrogen receptor-β than daidzein itself. Not all of the population is able to produce *S*-(−)equol, and the ability is linked to certain gut microbes (Virk-Baker et al. 2014). Some authors have described an association between a lower risk of breast pathology and *S*-(−)equol production in Asian women, but data on Western people are still scarce.

Other minor metabolites resulting from the oxidative phase I of daidzein and genistein are mainly 6-hydroxy-, 8-hydroxy-, and 3′-hydroxy-daidzein, as well as 6-hydroxy- and 3′-hydroxy-genistein (EFSA 2015). Chandrasekharan and Aglin (2013) did a review that can be consulted for more detail of the metabolization of isoflavones.

Briefly, the metabolization of isoflavones is enterohepatic, and detoxication is achieved mainly via cytochrome P450 (CYP) 1A2, although there is also passage via CYP2E1, 3A4, and 2C8 but to a lesser extent. The metabolization of estrogen follows a similar pathway. Genistein act as an inhibitor of the isoenzymes CYP1A2, 2C9, and 3A4. When genistein intake occurs concomitantly with intake of drugs that need these isoenzymes, potential drug interactions can occur, changing the therapeutic outcomes. However, if they will be consumed concurrently with

prodrugs that need these isoenzymes for biotransformation to their active forms, this process will not occur; therefore, the desired therapeutic effect will not occur or could be decreased. Therapy with anastrozole or tamoxifen for breast cancer can be affected by concomitant intake of these isoflavones.

These molecules, especially genistein, can have an affinity for β- and α-estrogen receptors (ERβ and ERα) of around 43% and 1%, respectively, giving a theoretical efficacy relative to estradiol (TERE) close to half of the activity of estrogen itself (Campos and Matos 2010). In white people this can induce an important increase in estrogenic activity (Ko 2014).

Another important matter is the relationship between the dose and the effect, which can be paradoxical. With regard to low doses, some authors have referred to results that showed induction of cancer cell proliferation in comparison with medium doses, which could present the potential to induce the negative feedback of estrogen production with aromatase inhibition. Doubts about safe use, especially at high doses, are also a big concern in Western countries (Ko 2014), and good assessments of potential toxicity are needed.

It is important to keep in mind that the food matrix and dose affect the absorption and metabolism of isoflavones in general and could change the end points of evaluations. In humans, the glucuronidated forms of isoflavones are detected in plasma and urine, achieve maximal plasma concentrations within 4–8 h, and are then eliminated from the body through the bile and kidneys, with a mean terminal elimination half-life of approximately 6–8 h (Chandrasekharan and Aglin 2013).

To ensure the reproducibility of clinical trials, the major important factors should be evaluated beforehand. First of all, the chemical composition of the extract is crucial, which means that all of the compounds need to be identified and quantified (Campos and Costa 2012). Host characteristics such as the metabolizer phenotype – which could be also linked to ethnicity, age, and the genotype – are also very important (Chandrasekharan and Aglin 2013).

Relevant data are now starting to become available to the scientific community and show these major differences and approaches that should be considered, but for the population in general, this information is not widespread.

Regarding all of these questions, deep reflection on the subject is needed. Nowadays, it is recognized that soybeans are an important source of protein used worldwide for various purposes including raising cattle and the majority of the animals needed for human consumption, such as chickens and pigs (Graham and Vance 2003), fish in aquaculture (Król et al. 2016; Casu et al. 2017), and even shrimps (Conklin 2003). Nonetheless, soy isoflavones that remain in the protein fraction are devoid of a nutritional characteristic *tout court*. Despite all of the research carried out aimed at establishing the nutritional value of isoflavones, they cannot be considered to have that function. They are not structural constituents like proteins, functional like lipids, or energetic like carbohydrates. As micronutrients, these structures are not fundamental for metabolic functions as vitamins or minerals. Even the antioxidant function attributed to them has not been shown to have a clear function in humans. As nutrients, at least for white people, their impact is ultimately negligible.

Despite all of the controversy referred to above, their ability to bind to estrogen receptors and mimic estrogen has been studied with the aim of replacing hormone

therapy, especially in postmenopausal women. As has been further described, in some circumstances they could be of benefit but also could result in risk, depending on the health status of the person and even depending on their ethnicity (Campos and Costa 2012). Postmenopausal women are an easy target in the marketing of these products, and great caution is needed to avoid side effects and chronic toxicity.

Therefore, consideration should be given to their consumption, especially because so far, no benefit of isoflavones has been proved in white women despite all the research already carried out. According to the EFSA's 2012 report, the EFSA Panel on Dietetic Products, Nutrition and Allergies concluded (in page 6) "*that the evidence provided was insufficient to establish a cause and effect relationship between soy isoflavones and maintenance of bone mineral density and reduction of vasomotor symptoms associated with menopaus*e." The US National Center for Complementary and Integrative Health (NCCIH; formerly known as the US National Center for Complementary and Alternative Medicine (NCCAM)) has also referenced this situation, saying "Current evidence indicates that it's safe for women who have had breast cancer or who are at risk for breast cancer to eat soy foods. However, it's uncertain whether soy isoflavone supplements are safe for these women" (NCCIH 2020).

Chen et al. (2019) did a review where they concluded that "In order to minimize study heterogeneity in future research, we urge standardization of the many variables involved in isoflavone trials, … Isoflavone aglycone content should be consistent across trials for better communication, prediction of therapeutic activity, and ensure reproducibility. In addition, outcome measures and study duration should be standardized, along with the metabolic profiles of participants." The same was claimed by Campos and Matos in 2010, when they purpose the determination of the Theoretical Efficacy Related to Estradiol (TERE) to assess previous data that predict the bioactivity of isoflavones through this model as a way to obtain a reproducible clinic trials.

Much research has been carried out in the last 30 years to gather enough data to appropriately assess the potential of soy isoflavones in cholesterol reduction, prevention of atherosclerosis and cardiovascular disease (CVD), chemotherapy, anti-inflammatory applications, and antioxidant activity, for instance. The interpretation of the data regarding the health potential of soy isoflavones must be done with great care. Even more important are the clinical trials (discussed later in the text) investigating isoflavones as potential treatments for atrophy and postmenopausal symptoms that are ongoing; these will either validate the previous findings or not, in order to allow safe use of these compounds.

7.4 Bioactivities (Animal Experiments/Animal Aspects)

Our literature search on animal experiments was mainly approached using the keywords "soy" and "isoflavones" and "animal." In the last 10 years, 225 papers were indexed in PubMed and 163 in the Web of Science, but only 81 and 73 of these, respectively, offered free full-text access. Of those, the sort order was changed to

"best match" and the first three papers were two from 2017 and one from 2013, which we describe below in more detail. All of the others are summarized in the text.

In the Web of Science, the main areas of publication were Nutrition Dietetics (117), Food Science Technology (55), Oncology (45), Biochemistry Molecular Biology (38), and Toxicology (30). The documents had a prevalence of articles (296) and reviews (83). The years of major publications in the field were 2007–2011.

Before discussing and presenting the animal experiments, it is important to undertake careful consideration of some of the experiments described below. For instance, we must not lose sight of the fact that isoflavones as plant ingredients can be considered antinutritional factors (ANFs).

Independent of the potential benefits, the assessment of the risk should bear in mind the following conclusions from Król et al. (2016): "Our results indicate that gut transcriptomic profiling provides a useful tool for testing the applicability of alternative protein sources for aquaculture feeds and designing diets with reduced impact of ANFs on fish health. Ultimately, understanding diet–gut interactions and intestinal homeostasis in farmed fish is important to maximize performance and to ensure that aquaculture continues to be a sustainable source of food for a growing world population." This issue was discussed with regard to fish, but it can also be studied in other animals, and similar data should be provided. The same question involves animals that we eat, but laboratory ones are used in experiments carried out with the intention of understanding the impact of these bioactive compounds. Laboratory animals are frequently fed with products containing soy protein and isoflavones. This is very important because these compounds have effects on the metabolism of these animals and may bias the interpretation of the results.

Below are summaries of animal experiments involving soy isoflavones and hormone-dependent tumors (HDTs). However, metabolism differs between humans and animals; therefore, the outcomes of animal studies may not be applicable to humans.

Kijkuokool et al. (2006) designed a study to determine the effects of genistein on *N*-nitroso-*N*-methylurea-induced tumorigenesis in adult female Sprague Dawley rats that were 30 days of age, with a bodyweight of 120–140 g. They verified median latency periods (to tumor growth) of 59.5 and 51 days for the vehicle and genistein, respectively. Genistein dosing was associated with increases in the cross-sectional tumor area, tumor multiplicity, metastases, and malignancy; serum 17β-estradiol levels were significantly higher in genistein-treated animals than in vehicle-tread controls during the first 2 months of treatment (the time when most of the rats developed tumors). The authors concluded that "supplementation of genistein at a dosage comparable to the isoflavone consumption in humans did not affect the reproductive system but resulted in enhancement of NMU [*N*-nitroso-*N*-methylurea]-induced tumorigenesis in adult female rats. Thus, the supplementation of soy isoflavone in premenopausal women may potentially potentiate the risk of breast cancer."

Breast and prostate cancers are prevalent HDT under close screening and study in WHO. All data that can be evaluated for prevention or for assistance with treatment

is important, but the extrapolation from animal data is not so accurate. For this reason, the major discussion in this chapter focuses on human trials.

With the increase in life expectancy, relief of peri- and postmenopausal symptoms in women is also a hot issue.

From the data analyzed, we emphasize the evaluation of two soy intervention trials by Maskarinec et al. (2017), who found little evidence of a difference in the response to soy foods between Asian women and non-Asian women.

In a study carried out in Mexico, Rodríguez-Landa et al. (2017) hypothesized that it might be possible to use the estrogenic activity of genistein to ameliorate anxiety in women undergoing surgical menopause, and so they investigated anxiolytic-like effects. The comparison was done with identical doses of genistein and 17β-estradiol (0.045, 0.09, and 0.18 mg/kg/7 days) in a surgical menopause model in rats, which did the elevated plus maze and locomotor activity tests 12 weeks after ovariectomy. The animals were adult female Wistar rats, weighing 200–250 g. The results were dose dependent. To support the extrapolation of the results to humans, the authors claimed that "these effects appear to be related to the activation of estrogen receptor-β (ERβ). Genistein has a structural conformation that is similar to 17β-estradiol, which allows it to be recognized by ERβ" (Rodrígues-Landa et al. 2017). Genistein does have affinity for ERβ (around 43.9% relative to estrogen itself) but also can be bound to ERα (around 0.86%) (Fokialakis et al. 2004). The input of these two links will contribute to the result when a correlation is done with estradiol that is able to bind 100% to both receptors (Campos and Matos 2010). The results indicate that genistein produces anxiolytic-like effects in ovariectomized rats, which highlights its potential therapeutic application.

However, in our point of view, this is not the only reason, as genistein has a configuration similar to a flavone type that is able to be a γ-aminobutyric acid (GABA)-A ($GABA_A$) ligand with antidepressant-like activity in the central nervous system. The same activity is also attributed to other flavonoids (Coleta et al. 2008) that are able to bind as benzodiazepine-like molecules to $GABA_A$ benzodiazepine receptors.

The data that are cited below are not directly related to animal experiments that could be the basis for human trials; the more relevant information is for humans that consume animals feed with diets including isoflavones, as is discussed at the end of this section.

To evaluate the effect of dietary genistein on the molecular mechanism of fatty liver syndrome (FLS), Lv et al. (2018) fed a high-energy, low-choline diet to hens, to establish an FLS model, in China. This pathology in hens is characterized by a large amount of fat deposited in the hepatocytes, resulting in a fatty liver, liver hemorrhage, and obesity. It can significantly decrease egg production and induce sudden death, resulting in major economic losses for the poultry industry.

The interest in the study cited above is the correlation of the results between the low and high doses of genistein utilized by the authors. In the experiment, hens treated for 64 days with 40 mg of genistein per kilogram had significantly increased transcriptional levels of gonadotropin-releasing hormone (GnRH) in the hypothalamus, while the same hormone was downregulated in hens fed 400 mg of genistein

per kilogram. These effects on GnRH explain the difference in the reproductive performance of laying hens with FLS and the negative effects of high-dose genistein, which could also be due to inhibition of tyrosine protein kinases and related effects. The authors noted that other studies on rodents also indicated that the effects of the genistein dose on production performance varied with sex, age, and endogenous hormone levels. They also showed that a low dose of genistein improved serum levels of estrogen, while a high dose had no significant effect. These data are important for animal studies in general.

Similar data have been reported by other authors. The optimum dietary dosage range for soy protein that contains isoflavones still needs to be clarified because many brands of laboratory animal feed contain this compounds, as was mentioned earlier.

According to the WHO Global Health Observatory (GHO), "ischemic heart disease, stroke, chronic obstructive lung disease and lower respiratory infections have remained the top killers during the past decade. Chronic diseases cause increasing numbers of deaths worldwide. Diabetes caused 1.6 million (2.8%) deaths in 2015, up from 1.0 million (1.8%) deaths in 2000. Deaths due to dementias more than doubled between 2000 and 2015, making it the 7th leading cause of global deaths in 2015. Injuries continue to kill 5 million people each year. Road traffic injuries claimed about 3700 lives each day in 2015, about three-quarters of whom were men and boys."

As noted above, dementia is an increasing menace to health in the twenty-first century, probably with more patients in high-income countries, and much research is being carried in this field. Flavonoids, including genistein, are even being screened as a possible treatment for Huntington's disease (Pierzynowska et al. 2018). Although dementia is a complicated disease with no cure, hypotheses regarding use of these substances to alleviate some of the symptoms are promising. Therefore, the results presented in this report merely provide a basis for the use of genistein in further studies.

7.5 Benefits (Function in Humans/Human Studies)

Since Axelson et al. (1982) first published the finding of equol in human urine, various publications regarding isoflavones, from different sources, including the same team (Setchell et al. 1984; Setchell and Cassidy 1999), have speculated about the importance of phytoestrogens in hormone-dependent diseases. Notwithstanding this and all of the recent findings about the therapeutic potential of isoflavones, when work on phytoestrogens first started to be published and equol was found in the urine of sheep in Australia, this was associated with infertility of the sheep (Bennetts et al. 1946). In fact, soybeans have become one of the more widely consumed foods that contain isoflavones, although these compounds are in fact widespread in nature in many other plants, as was noted earlier. Other sources of soy dietary isoflavones include soy-rich foods such as tofu, miso, and soy milk, as well as soy-based ingredients – for instance, soy protein concentrate and isolate, soy

flour, and a range of isoflavone-rich supplements. Within Europe, dietary intakes of isoflavones has generally been very low (<5 mg per day), but between 2002 and 2012 an increase in that intake has been verified, together with its impact of estrogen-like activity in some genetic groups, such as white people. This could be dangerous, given the potential of estrogenic-like activity and hypothyroidism (Campos and Costa 2012). The same situation should probably be evaluated in other ethnic groups such as African Americans because in the USA the intake of soy and soy-derived products is too high and the relevant data are too scarce.

Setchell and Cassidy (1999) and Setchell et al. (2002) described dietary isoflavones as having relevance to human health because of their biological effects; however, at that time, ethnic differences or the possibility of inducing side effects or even chronic toxicity were not evaluated.

From data collected in a Japanese population, it has been possible to draw interesting conclusions intrinsic to this target population (Ueshima 2007). The fact that this population consumes soy and has little propensity for HDTs may have a direct relation, given the low plasma concentration of the hormones involved. These results have become known as the "Japanese paradox" and have induced people around the world to consume soy and soy derivatives rich in isoflavones such as genistein, daidzein, and glycitein, and their respective glycosidic forms (genistin, daidzin, and glycitin) (Nakamura and Ueshima 2014). A large number of studies have attempted to demonstrate that soy consumption decreases the risk of developing several chronic diseases (in particular, CVD, cancer, and osteoporosis) and relieves climacteric symptoms, but the estrogen impact is not well understood yet, and a huge difficulty remains when data are compared, given the often controversial end points of the studies. In Campos and Matos (2010) and in Campos and Costa (2012), a theoretical calculation was suggested for application to the different isoflavones under study in a way to achieve accuracy of the results.

Up until now, however, the majority of the benefits have not been proved safe. The difficulty is to get a good correlation comparing the ethnicities under study and quantification of the various isoflavones in the products that are involved; all of this can give different outputs for benefits and risks (Campos and Matos 2010).

From the data collected and analyzed in this review, we summarize below the main topics that are discussed: CVD, HDTs, breast and prostate cancer, peri- and postmenopause, thyroid outcomes, and recent studies proposing isoflavones in Huntington's disease and sleep disturbance.

It is evident that the end points have been mostly the same throughout the chronology, and evaluation of secondary or side effects has been scarce. Five publications evaluated breast outcomes (breast density, epithelial proliferation, and mammographic abnormalities), ten publications evaluated uterine outcomes (endometrial thickness), and four publications evaluated thyroid outcomes (circulating thyroid hormones). Only one study reported adverse events. Statistical differences between isoflavone and placebo groups in the outcomes mentioned below were not reported.

Recently, Namasi et al. (2018) did a systematic review of cohort studies on the association between consumption of soy products and mortality from all causes,

CVD, and cancer. This aimed to clarify the association of soy intake with total and cause-specific mortality. Briefly, from the data that were analyzed, the authors verified no significant association between a high intake of soy products and all-cause, CVD, and cancer mortality. However, what was highlighted was the need for further studies to clarify the association between soy product intake and the risk of mortality. Summarized information and conclusions are given in the following sections.

7.5.1 Cardiovascular Disease

Cardiovascular diseases are defined by the WHO as "disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions." Every year, 17.9 million people die from CVD, accounting for 31% of global deaths.

The Global Burden of Diseases, Injuries, and Risk Factors updated snapshot of the state of CVD over the last 25 years reaffirms that it continues to be a significant public health concern worldwide despite impressive advances in the technical capacity for preventing and treating CVD. The main risk factors (hypertension, dyslipidemia, and diabetes) have become a collective concern for higher- and middle-income countries (Basu et al. 2019).

Data from the WHO point to 1.1 billion adults having raised blood pressure, which is detrimental to good heart health, despite the availability of drugs that can be used to control this disorder.

Isoflavones, like most flavonoids, are capable of inducing vasodilation and lowering blood pressure via this pathway.

Taku et al. (2010) published data from a systematic review and meta-analysis of randomized placebo-controlled trials (14 trials totaling 789 participants), where they presented the effects of soy isoflavone extracts on blood pressure in adult humans. The mean daily ingestion of soy isoflavones (as aglycone equivalents) was 25–375 mg for 2–24 weeks. The most significant result showed a decrease in systolic blood pressure (SBP) by 1.92 mmHg (95% confidence interval −3.45 to −0.39, $P = 0.01$) in comparison with the placebo (heterogeneity $P = 0.39$, fixed-effects model), but no dose–response relationship was observed. These data were collected from adults with normal blood pressure and prehypertension. This suggests a greater effect in studies lasting longer than 3 months, in Western populations, at lower doses, and in studies with a lower risk of bias. The authors also verified from the data collected that soy isoflavones did not affect diastolic blood pressure (DBP).

Jackson et al. (2011) also showed that isoflavones can contribute to hypertension control by inducing vasodilation. They can improve brachial artery flow because of the interaction with the estrogen-response element of genes related to endothelial nitric oxide synthase, increasing endogenous nitric oxide production and thus inducing vasodilatation.

Carmignani et al. (2014) aimed to verify the effect of a soy dietary supplement and a low dose of hormone therapy on the main cardiovascular health biomarkers in

a 16-week, double-blind, randomized, placebo-controlled trial. (The trial was registered at the Brazilian Clinical Trials Registry (Registro Brasileiro de Ensaios Clínicos), study number RBR-76mm75.) The study included 60 participants recruited from two menopause outpatient clinics at the Women's Integrated Health Care Center at the University of Campinas (UNICAMP) in Campinas (São Paulo) and at the Leonor Mendes de Barros Maternity Hospital in São Paulo (Carmignani et al. 2014). The main goal was to assess the effect of soy dietary isoflavone supplementation on clinical biomarkers of cardiovascular health and serum changes in the lipid profile and fasting glucose levels. The isoflavone intervention consisted of 20 g per portion of a food powder containing 12 g of soy protein and 45 mg of total isoflavones (26.5 mg aglycones), to be mixed with 200 mL of any beverage. The soy intervention contained approximately 8 mg of total daidzein, 15 mg of total genistein, and 3.5 mg of total glycitein.

If the theoretical efficacy (TE) and the TERE were calculated as proposed by Campos and Matos (2010), in accordance with the formula cited above (for the three isoflavones daidzein, genistein, and glycitein), as is exemplified in Table 1, the understanding of the outcomes for the results obtained by Carmignani et al. (2014) would be more clear. As is shown in Table 1, the estrogen-like impact of mixing these isoflavones has a TERE of 1/15, corresponding to 14.85% of estradiol activity, which is very low. Probably, this is under the therapeutic value needed to achieve a good outcome. The values used for estrogen affinity among the estrogen receptors in this calculation came from Fokialakis et al. (2004). If the researchers had had this information before the clinical trial, the prevision for the outcomes (estrogen affinity for both receptors) would have been more effective.

In fact, from data collected in the clinical trial, the authors concluded that "the use of dietary soy supplement did not show any significant favorable effect on cardiovascular health biomarkers compared with hormone therapy." The study was performed in Brazil with no ethnic information given, but, in general, its results are in line with the low 1/15 activity if the TERE is calculated as above.

Table 1 Theoretical calculation of the efficacy of the two mixes of isoflavones described in the essay cited by Carmihnani et al (2014), using the estrogen receptor (ER) affinity–binding values for compounds including daidzein and genistein

		Receptor type	
	Intake (mg/day)	ERα	ERβ
Daidzein	8	0.00248	0.0016
Genistein	15	0.129	6.585
Glycitein*	3.5	Very low/irrelevant	
Total	26.5	0.13148	6.5866
TE	6.71808	(= 0.13148 + 6.5866)	
TERE	1/15 of the theoretical activity of estradiol (14.85% of estradiol activity)		

TE theoretical efficacy, *TERE* theoretical efficacy relative to estradiol
*very now bioactivity which is irrelevant for the total.

In Japan, Nakamura and Ueshima (2014) evaluated the Japanese paradox in correlation with cholesterol levels and coronary stroke. One of the studies they analyzed was performed by Shimazu et al. (2007), who prospectively assessed the association between dietary patterns and CVD mortality in 40,547 Japanese men and women aged 40–79 years without a history of diabetes, stroke, acute myocardial infarction, or cancer at the baseline in 1994. After adjustment for potential confounders, the Japanese dietary pattern score was associated with a lower risk of CVD mortality despite the fact that the Japanese dietary pattern appeared to be related to higher sodium intake and a high prevalence of potential hypertension. Soybeans were one of the relevant foods, among others, and no correlation was established between these parameters.

Ramdath et al. (2017), from Canada, reviewed evidence on the cardiovascular benefits of nonprotein soy components in relation to known CVD risk factors such as hypertension, hyperglycemia, inflammation, and obesity beyond cholesterol lowering. Although the evidence suggested that nonprotein soy constituents improved markers of cardiovascular health, further careful studies are required to confirm these effects.

7.5.2 Hormone-Dependent Tumors: Breast and Prostate Cancer

Cancer is the second leading cause of death globally and was responsible for an estimated 9.6 million deaths in 2018. Globally, about one in six deaths is due to cancer, with approximately 70% of these deaths occurring in low- and middle-income countries. Dietary risks such as low fruit and vegetable intake contribute to one third of deaths from cancer. That is why much research is focused on foods and their respective bioactive molecules that can help to prevent and/or treat such disease.

Among the most common cancers worldwide that cause death, breast cancer (2.09 million cases) and prostate cancer (1.28 million cases) are in the top five, being first equal with lung cancer (2.09 million cases), followed by colorectal cancer (1.80 million cases), skin cancer (nonmelanoma cancer) (1.04 million cases), and stomach cancer (1.03 million cases) (Bray et al. 2018).

The etiology of these cancers is diverse and sometimes controversial, as is the case for breast and prostate cancers, which are the most hormone-dependent tumors, with estrogen and androgen being the key drivers for both. In the USA, for example, breast cancer rates are extremely high overall, largely because of the high incidence of estrogen receptor–positive cancers among older women. Although the causes of HDTs are more broad, implementation of existing evidence-based prevention strategies could be a way to prevent them and, in certain circumstances, to help treat them.

Some years ago, the data indicated that the low rate of prostate cancer in Asians could well be associated with soy intake (Cook et al. 1999). Yan and Spitznagel (2009) systematically reviewed 15 epidemiological publications on soy consumption and nine studies on isoflavones in association with prostate cancer risk in a

multiethnic population. They found a good correlation, suggesting that consumption of soy foods is associated with a reduction in prostate cancer risk in men. However, the majority of the epidemiological studies on consumption of fermented soy foods in association with prostate cancer risk in men were from a Japanese population.

A more recent study using Status in Surveillance, Epidemiology, and End Results (SEER) data suggested that the quality of race data in cancer registries is excellent for white people, black people, and Asians/Pacific Islanders, and it is substantial for Hispanics and American Indians/Alaska Natives, but considerable underreporting has been shown for other ethnicities (Máire et al. 2016). Thus, reported differences in rates among racial and ethnic populations should be interpreted with caution. Information in Máire et al. (2016) is also reporting of prognostic measures that may influence treatment, such as the relationships between a 21-gene prognostic signature for breast cancer and the frequency of chemotherapy treatment, and the intensity of follow-up for low-risk prostate adenocarcinomas managed with "watchful waiting."

Soy isoflavones are among the more controversial molecules involved in the research being discussed. They have been suggested to be protective against cancer (genistein, for instance, can act as an aromatase or 17β-hydroxysteroid dehydrogenase inhibitor, decreasing estrogen and testosterone, respectively) or may act as proliferation inducers for estrogen-like activity. Moreover, they can modulate the activities of key enzymes in the biosynthesis and metabolism of steroid hormones, acting as agonists or antagonists at estrogen receptors, pregnane X receptors, and constitutive androstane receptors (Cassetta et al. 2017).

Evidence was reported by Shu et al. (2009) suggesting a possible benefit in survivors of breast cancer related to soy consumption. This analysis was carried out on data from 5042 Asian women in China in the Shanghai Breast Cancer Survival Study; therefore, its results cannot be extrapolated to white women, Africans, or African Americans, for instance. In addition, the data were only observational, and the consumption of soy-based foods (which, incidentally, has been practiced by this population for generations) was assessed only through the use of questionnaires, which also included other foods such as meat, fish, and vegetables. This study included many other favorable variables, which also contributed significantly to the "outcomes" – for example, additional treatments with tamoxifen, vitamin supplements, consumption of "tea" (supposedly from *Camellia sinensis*, although this was not specified), and physical activity. In addition to all of this, the authors also mentioned other types of food, such as crucifers, as important contributors to the final outcome. The authors attributed the survival of women with breast cancer in the study to all of these factors, not just the consumption of soy or soy-related products (Shu et al. 2009).

To further clarify this issue and other similar situations, a meta-analysis was conducted by Dong and Qin (2011) from the Department of Nutrition and Food Hygiene at the University of Soochow in China. They concluded that the intake of soy isoflavones may be associated with a reduced risk of breast cancer in Asian populations but not in the West.

Regarding the absence of relevant data on low doses of genistein and possible enhancement of the proliferation of HDTs, Steinberg et al. (2011) conducted a clinical trial that clearly demonstrated that a dose of 10–15 mg/daily brought no benefit for healthy postmenopausal women. As for the "risk," one case of breast cancer and one case of endometrial cancer were verified in the study. Despite the fact that these cancers can occur in this age group, the authors reported that in fact isoflavones may have contributed to these events. This is one of the most problematic situations, since the amount of genistein that binds to ERα is low in this case but may be enough to cause prestimulation of tumor cells, as has been reported in several studies. The authors further suggested that there is a possible small risk to healthy women after menopause. The evaluation was performed with around 80–100 mg of isoflavones, which seems to be a high dose, but the amount of the more active isoflavone, genistein, was minimal (10 and 15 mg in the two groups under study). Daidzein just might have had some relevance but only if the women were "equol producers," which was not the case with any of them (this feature is more common in Asian populations). The impact on the thyroid was small but visible, although the doses were low with respect to preparations currently on the market, since the investigators just wanted to mimic a vegetarian diet with the inclusion of soy.

In a previous study where the exposure to isoflavones was greater than in the work cited above, Unfer et al. (2004) evaluated a similar group of women for 5 years. In this study the amount of genistein (the more active isoflavone), as an example, corresponded to 67.5 mg/day. The authors noted that there was an increased risk of endometrial hyperplasia, which had also been reported elsewhere (Hu et al. 2003).

The importance of evaluating the respective doses of those bioactive compounds is crucial for assessment of the effects of different levels in women and to compare clinical trials with each other. According to the TERE calculation proposed by us some years ago (Campos and Matos 2010), the result would be 29.63% affinity for ERβ and 0.585 for ERα, given 30.3% activity, which relative to estradiol would be a little below 1/3. This already represents considerable estrogenic activity.

In their meta-analysis, Chen et al. (2014) noted that isoflavone intake had a possible effect in reducing the risk of HDTs in women of ethnicities associated with Asian countries, but there was no evidence to suggest that the same consumption could be similarly effective in women of other ethnicities from Western countries.

Consistently reported risk factors for prostate cancer are age, race/ethnicity, and family history, and the risk increases rapidly among older men. It is the most commonly diagnosed cancer among men in the Western world, and African American men have the highest rates in the USA (WHO data).

If prostate cancer treatment is started early, the prognosis is good, but the treatment often leads to side effects such as urinary and erectile dysfunction. Therefore, an alternative strategy is very important because in certain cases, androgen deprivation therapy, as the standard of care, fails.

The enzyme 5α-reductase, which converts testosterone into dihydrotestosterone, has been involved in breast and prostate cancers. Therefore, breast carcinomas that

are positive for 5α-reductase type 1 may partly maintain some androgen regulatory mechanisms (Li et al. 2009) and be partly associated with the proliferation effect of progesterone in breast cancer cells.

The relevance here is that one of the anticancer mechanisms proposed for genistein is linked with inhibition of 5α-reductase activity in prostate tissue. Nevertheless, even in this case, the results are controversial (Lou et al. 2005). Only limited data from intervention studies in human are available, and the results are still unreliable.

The steroid 5α-reductase type 2 (SRD5a2), which is very important in androgen metabolism, has two polymorphisms in its gene (*SRD5A2*) – V89L (rs523349) and A49T (rs9282858) – which have been studied for associations with prostate cancer risk, with conflicting results. To provide a more precise estimation of these two polymorphisms and prostate cancer risk, Li et al. (2013) performed a meta-analysis using all published case–control studies of prostate cancer published since January 1995. However, prostate cancer is a biologically heterogeneous disease with variable molecular alterations, and the results were not conclusive.

Yan and Spitznagel (2009) reported that the inverse association between soy consumption and prostate cancer risk found in epidemiological studies was supported by animal studies. The data were related to dietary soy protein and soy phytochemical extracts as the main sources of compounds that inhibited experimentally induced prostate tumorigenesis. However, one study also showed that soy protein increased prostate tumor growth in an androgen-independent model. Genistein, as has already been noted, is the isoflavone that is more implicated in prostate cancer prevention and even as a possible treatment on the basis of its inhibition of tumor development in animals and proliferation of prostate cancer cells in cultures.

Zhang et al. (2016), from China, did a review with a critical discussion of some issues correlated with the protective effect of isoflavones against the development of prostate cancer. As their main conclusion, they stated that "Isoflavones play a protective role against the development of prostate cancer. However, careful consideration should be given when isoflavones are used in the prevention and treatment of prostate cancer." Briefly, the authors did not find data supporting an important role for isoflavones in prostate-specific antigen level reductions in cancer patients or healthy men. As was discussed previously in this text, the effect of isoflavones on sex hormone levels and on the risk of this cancer could be determined by equol-converting bacteria in the intestine, but this is directly correlated with specific polymorphic variations and the concentrations of isoflavones. Another important point, which has already been discussed here, is that the intake of various types of phytoestrogens with lower concentrations in the daily diet may produce synergistic effects against prostate cancer. Moreover, prostate tissue may concentrate isoflavones to potentially anticarcinogenic levels. In addition, it should be noted that isoflavones may act as agonists in prostate cancer.

The discussion is wide ranging because in some randomized, controlled trials on the role of isoflavones in men with prostate cancer, the results have been inconsistent as to whether isoflavones can lead to cancer progression.

7.5.3 Perimenopause and Postmenopause

The impact of soy isoflavones differs depending on ethnicity, and in Europe, different groups have studied this issue to do a risk assessment of the increased intake of such compounds by the white population.

In 2015, an external scientific report titled *Preparatory Work to Support the Risk Assessment for Peri- and Postmenopausal Women Taking Food Supplements Containing Isolated Isoflavones* was published. The authors (Tijhuis et al. 2015) issued the following disclaimer: "This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority." The text stated that "food supplements targeted at peri- and postmenopausal women typically provide a daily dose of isoflavones in the range of 35–150 mg/day." In addition, it was emphasized that "the background exposure from the diet in the general European population was estimated to be lower than 1 mg/day, whereas in consumers of soy-based foods it could be higher." The panel concluded that "it was not possible to derive a single health-based guidance value for the different preparations in post-menopausal women. However the doses used in the intervention studies and their duration could serve as guidance for the intake of food supplements." From the data collected, the authors mentioned the following, among their main conclusions: (a) "isoflavones may interact with the synthesis of thyroid hormone"; (b) "the human data did not support the hypothesis of an increased risk of breast cancer from observational studies nor of an effect on mammographic density nor on proliferation marker Ki-67 expression in interventional studies"; (c) "no effect was found on endometrial thickness and histopathological changes in the uterus up to 30 months of supplementation with 150 mg/day of soy isoflavones"; and (d) after 60 months, some nonmalignant histopathological changes were reported. Most of these four points need to be clarified to assure a safe mode for isoflavone intake by white population.

One of the most problematic symptoms in postmenopausal women is hot flashes, which have been reported in approximately 70–80% of US women of menopausal and perimenopausal age, in comparison with approximately 10–20% of Asian women. It has been estimated that the average estimated blood concentration of the soy isoflavone genistein is approximately 25 ng/mL in Asian women but only 2 ng/mL in US women. From the review and meta-analysis by Li et al. (2015), it was concluded that soy isoflavones show slight and slow effects in attenuating menopausal hot flashes in comparison with estradiol. The authors measured the efficacy of soy isoflavones and conducted a systemic literature search to build a time–effect model for placebo and soy isoflavones in treating menopausal hot flashes. In this model, it was hypothesized that the placebo and soy isoflavone effects would vary with time and reach a plateau. Therefore, the effect profiles for placebo and soy isoflavones were described with a sigmoid maximum effect (E_{max}) model, with time considered as an independent variable. From the data analyzed under these

parameters, the authors achieved a time course for the reduction of hot flashes after administration of the placebo and soy isoflavones. The data that were collected showed a maximal percentage change (in terms of reduction of hot flashes) with soy isoflavones of 25.2% after elimination of the placebo effect, amounting to 57% of the maximum effects of estradiol (estradiol E_{max} 44.9%). However, they also found that a time interval of 13.4 weeks was needed for soy isoflavones to achieve half of their maximal effects, which was much longer than the interval for estradiol, which was only 3.09 weeks. Similarly, treatment intervals of 12 weeks are too short for soy isoflavones, which require at least 48 weeks to achieve 80% of their maximum effects. These data are relevant to understanding the problem and optimizing the design of possible clinical trials. In Table 1, details are provided about the concentration of the different isoflavones included in the placebo-controlled trials under analysis. A further study should be done using the determination of the TE and TERE proposed by Campos and Matos (2010) to complete the approach started by Li et al. (2014).

7.5.4 Thyroid Function

An increase in the risk of hypothyroidism in peri- or postmenopausal white women is a very important aspect and should be discussed with respect to the consumption of soy and products containing isoflavones. The results reported by Sathyapalan et al. (2011) have proved to be interesting because these authors evaluated the effects of soy isoflavones in white women with "subclinical hypothyroidism." This clinical situation is widespread, particularly in women with a large number of anti-thyroperoxidase antibodies, which are often found as biomarkers in the development of Hashimoto's thyroiditis. During its progression, this leads to destruction of the thyroid and requires lifelong replacement therapy with levothyroxine, which is one of the five most widely consumed drugs worldwide, precisely because of the high prevalence of this disease in women. In a randomized, double-blind study, it was estimated that the risk of hypothyroidism was three times higher with isoflavone intake from 30 g of soy protein, containing 16 mg of total isoflavones, corresponding to 8.64 mg of genistein, 5.6 mg of daidzein, and 1.92 mg of glycitein. What was more alarming in this study was that it gave support to the existing concerns about the consumption of isoflavones in the short and long terms, resulting in inadequate synthesis of hormone due to thyroid inhibition of thyroperoxidase, together with increased urinary excretion and fecal bile displacement of T4 from transthyretin. The persons involved in this study had very good iodine supplementation, which was indicated by elevated urinary excretion of iodine; this is not the case for most of the world's population. This excretion of iodine was not changed by isoflavone intake. In this study, genistein levels, which is the one that has shown more inhibition among isoflavones, to peroxidase thyroxine are in very low dose (8.64 mg) in comparison with what is found in extracts available on the market (Campos and Costa 2012), but this is a dose that can probably be found in dairy products.

Thus, a comparison of this impact is complex to evaluate, and the risk may be higher that we suppose. Anyway, clinicians should be aware of this relevant information to ensure better diagnosis of this pathology.

7.5.5 Sleep Status

Good sleep is crucial for good health. Since estrogen modulates sleep duration and quality, Cui et al. (2015) hypothesized that isoflavones would have a beneficial effect on sleep status in a way similar to estrogen. They conducted a cross-sectional study to examine the relationship between daily isoflavone intake and sleep status in Japanese subjects. To explore this idea, they studied 1076 Japanese adults aged 20–78 years, and they assessed the daily isoflavone intake by means of a brief self-administered diet history questionnaire. Sleep was evaluated using a self-reported questionnaire too. From the data obtained, the authors concluded that "higher daily isoflavone intake was positively associated with optimal sleep duration and quality in a Japanese population. This finding suggests that daily isoflavone intake may have a potentially beneficial effect on sleep status."

7.6 Epidemiological Studies

Bennetts et al. (1946), who were trying to identify the cause of a specific sheep-breeding problem in Western Australia, discovered phytoestrogens, as mentioned above. They implicated equol, a metabolite of daidzin that is an isoflavone existing in subterranean clover pastures, as the toxic molecule. Almost four decades later, equol was identified in human urine and related to soy intake in Asian diets. Further, it was found to be implicated in possible prevention of HDTs, CVD, and osteoporosis. Much research has now been published, but with conflicting data. In a short letter to the editor of the *American Journal of Clinical Nutrition*, Messina (1995) presented his point of view, stating that the isoflavone intake by Japanese was overestimated. He explained that Setchell and Cassidy (1999) estimated a Japanese daily per capita isoflavone intake of 150–200 mg, whereas his estimate was much lower. The outcomes discussed in these publications would therefore be different. This is one of various examples in the literature on this issue.

At that time, the world was focused on the "French paradox" based on data collected in a French population, which suggested that ingestion of antioxidants, mainly from red wine and alcohol itself, could be a panacea for cancer prevention and CVD. According to data from the world's largest study of heart disease, conducted by the WHO in 21 countries and including 10 million men and women, the French heart disease statistics appeared to have been underestimated and the French paradox overestimated. The French rate of heart disease was actually similar to those of the Italians, Spanish, and Germans (mainly those in Southern Germany) but still lower than those of many other countries (Campos and Costa 2012).

Other researchers looking in a different way for similar phenomena performed epidemiological studies in Asian populations in the 1990s that revealed a possible relationship between ingestion of soy, prevention of HDTs, and a lower incidence of climacteric symptoms in postmenopausal women.

These results, known as the Japanese paradox (based on data collected in a Japanese population), induced Western people to consume soy and soy derivatives rich in isoflavones such as genistein, daidzein, and glycitein, and their respective glycosidic forms (genistin, daidzin, and glycitin). A large number of studies have attempted to demonstrate that soy consumption decreases the risk of developing several chronic diseases – in particular, cancer, osteoporosis, and CVD – and relieves climacteric symptoms. At this stage, however, the majority of these postulated benefits should be considered with caution, as has been demonstrated in this text.

The two epidemiological studies described above were mainly focused on prevention of CVD and cancer, using phenolic compounds as the targets for this bioactivity. The first one, regarding the French paradox, was flawed and disregarded an important side effect – cirrhosis – in people with an increased intake of wine over a period of decades. With regard to the Japanese paradox, the scientific community has started to gather sufficient data to suggest that this concept needs to be assessed with caution to prevent a future failure mainly associated with the estrogen-like effect (Campos and Costa 2012).

So far, even 30 years later, with epidemiological studies suggesting that breast cancer rates are low in populations that consume soy, the following statement from Strauss et al. (1998) remains valid: "Epidemiological studies suggest that diets rich in phytoestrogens (plant estrogens), particularly soy and unrefined grain products, may be associated with low risk of breast and prostate cancer. It has also been proposed that dietary phytoestrogens could play a role in the prevention of other estrogen-related conditions, namely cardiovascular disease, menopausal symptoms and post-menopausal osteoporosis. However, there is no direct evidence for the beneficial effects of phytoestrogens in humans. All information is based on consumption of phytoestrogen-rich diets, and the causal relationship and the mechanisms of phytoestrogen action in humans still remain to be demonstrated. In addition, the possible adverse effects of phytoestrogens have not been evaluated. It is plausible that phytoestrogens, as any exogenous hormonally active agent, might also cause adverse effects in the endocrine system, i.e. act as endocrine disrupters."

The major problem in these meta-analytical approaches correlating dietary patterns in population studies is that they cannot be reproduced in other study populations. Consequently, nutritional studies using factor analysis have reported quite different patterns, even when dietary patterns have been successfully linked to disease protection. The converse is also true in these evaluations when a good correlation can be established between feeding behavior and specific estimates regarding relative risks or mortality. The studies are consequently not reproducible nor comparable. These scenarios of unreproducibility limit the significance of the a posteriori pattern analysis approach in epidemiological research (Nakamura and Ueshima 2014).

7.6.1 Evaluation and Management of Dietary Isoflavones in Different Ethnic Populations

Genetic differences between people from different ethnic groups should be evaluated carefully when the goal of the epidemiological study is to provide guidelines to improve the consumption of a product to avoid/prevent a disease. For example, African and white women are genetically different from Asians, as well as from other ethnic groups, as has been discussed. Genetic variants are closely linked to metabolism, via CYP, and the various isoforms that constitute it, with a direct bearing on the specific diets, typical of each ethnic group, that influence existing polymorphisms. The existence of these metabolic changes influences the activity of these isoenzymes even when they are exposed to the same substrate in the same metabolic process.

Isoflavone content is not restricted to soybeans; isoflavones can also be found in other foods such as adzuki beans, alfalfa, green peas, chickpeas, lentils, lupins, and peanuts, among others, as has been discussed above (Ko 2014). Soybeans are used in various traditional cuisine dishes all over the world; the amounts of isoflavones found nowadays in some products should be assessed to ensure that some diseases associated with estrogen disruption and/or thyroid dysfunction are not being caused by the increase and subliminal amounts of these compounds in Western diets.

With the increased use by Western populations of Asian medicinal plants – among which are some that contain isoflavones, such as *Astragalus* and *Angelica* roots, *Cimicifuga*, and kudzu – the impact in different ethnic populations should be assessed to evaluate the risks as soon as possible. Many of these issues are being discussed on the basis of the relevance of the research developed in the field; however, the broader end points of these bioeffects remain unclear (Wuttke et al. 2007; EFSA 2012; Campos and Costa 2012; Ko 2018).

As a simple example, in the case of soy isoflavones, estrogen and genistein have a similar metabolic pathway involving CYP1A2. In different ethnic groups, there are polymorphisms in that isoenzyme (Testa and Krämer 2008), and the cross-effect of the joint metabolism of these substances should be evaluated carefully. The polymorphic variation in this isoform is mainly due to genetic factors (39–72%). Asians, for instance, have several polymorphisms in *CYP1A2* that affect efficacy and toxicity in the metabolism of various drugs and catalytic activation of carcinogenic heterocyclic amines. This isoform can be induced by tobacco smoke and can be inhibited by grapefruit juice or by genistein itself. Caffeine metabolism occurs via the same route. Although there are different polymorphic forms, in white people there is a predominance of isoform *CYP1A2*1F*, which has been associated with increased levels of carcinogen formation and thus tumor induction.

From our point of view, these data should be integrated into the selection of patients for randomized clinical trials. Genetic information about the patients involved is crucial for full understanding of the data collected and the results obtained.

It is important to note that these genetic modifications in different ethnic groups are also linked to age and gender. In women, they are connected to the age at which

menopause arises and symptoms related to low estrogen that occur during this period of women's lives.

In Campos and Costa (2012), data were correlated with regard to the genes involved in the metabolism of steroid hormones and their association with hormonal levels and symptoms related to menopause. Briefly, the populations involved in some of the studies were African Americans and European Americans. The genotypes evaluated were catechol-*O*-methyltransferase (*COMT*); *CYP1A2*, *CYP1B1*, *CYP3A4*, and *CYP1A1*; and CYP19 sulfotransferase (*SULT1A1*) and *SULT1E1*; the related hormone levels were also evaluated. The results showed that European American women with the *SULT1E1* variant had low levels of dehydroepiandrosterone sulfate and lower levels of estradiol sulfate dehydroepiandrosterone and testosterone than women without this variant allele. Also, in this group, *SULT1A1*3* was associated with symptoms related to hot flashes and depression. In African American women, the *CYP1B1*3* genotype was associated with hot flashes and *CYP1B1*4* was found to be associated with depressive symptoms. Changes in *CYP1A2* genotypes were associated with hot flashes in postmenopausal women. The fact that Asians have different CYP polymorphisms may cause different effects in this period of life with respect to climacteric symptoms. Thus, the approach for preventive treatment of the symptoms associated with this situation should be adapted to women's ethnicity to be more beneficial and avoid risk where possible. The discussion of the points noted above cannot be separated with regard to isoflavone supplements or food products, because the risk associated with their intake by white people remains the same.

CYP isoforms are important in the metabolism of endogenous hormones and can cause changes in their quantity and essentially in the type of metabolites produced, but those are still poorly studied. Polymorphic changes are due primarily to the foods that individual ethnic groups have been exposed to for generations, their environment, and their way of life. Over the centuries, Caucasians have not been exposed to soy and/or isoflavone-rich foods, unlike Asians, and in Europe the isoflavones from various plants have been considered antinutrients and not recommended even for animal consumption.

For white people, caution should be exercised especially when these products are advertised as having beneficial health impacts for them, once isoflavones and/or all the products that contain those bioactive compounds, because for this ethnic group the risk assement was not did over a sustained period, that allows to say that they are safe.

Virk-Baker et al. (2014) designed a study to evaluate the relationship of the prevalence of breast cancer, ductal hyperplasia, and overall breast pathology with the capacity to be an *S*-(−)equol producer, as compared with nonproducer postmenopausal women undergoing a breast biopsy. A cross-sectional study design was used to evaluate the impact of supplementation of the participants' usual diets with one soy bar per day for three consecutive days. The results showed that the threshold value of −1.60 used for defining *S*-(−)equol status in the population under analysis was comparable to the ratio of −1.75 previously published by Setchell and Cole (2006). This study was the first to test the *S*-(−)equol-producing status of African

American women with a relatively large sample size ($n = 51$). Of the African American participants, 13.72% (7 out of 51) were *S*-(−)equol producers as compared with 19.56% of white Americans (18 out of 92), but the difference was not statistically significant. Briefly, the authors observed no "associations between *S*-(−) equol producing status and breast pathology, hyperplasia, or breast cancer. Based on our results of null associations, we rejected our hypothesis of lower prevalence of ductal hyperplasia, breast cancer, or overall breast pathology among *S*-(−)equol producers as compared to non-producers" (Virk-Baker et al. 2014).

The lower prevalence observed among African American women in the study described above was similar to results published by Hedlund et al. (2005) regarding white men who were long-term low-soy consumers.

Further studies are needed to confirm the observed lower prevalence of the *S*-(−) equol phenotype among postmenopausal African American women in order to further understand the dietary or lifestyle factors responsible for the observed lower prevalence in this group. To be more precise, the data that point to a protective effect in prevention of breast cancer associated with consumption of isoflavones (usually from soybean) and to *S*-(−)equol-producing status imply continuous consumption and not only a short-term consumption.

The Indian Menopause Society has projected that by the year 2025, there will be 103 million menopausal women in India, and data on the relief of symptoms are still scarce. At the gynecology outpatient department of a teaching hospital in Northern India, Ahsan and Mallick (2017) did a questionnaire-based, observational, non-randomized, prospective, open-labeled pilot study over a period of 1 year (October 2012–2013). The study involved 29 perimenopausal women and 21 postmenopausal women. The baseline was determined by a previous determination of the Menopause Rating Scale (MRS) on a specific questionnaire. Both groups started treatment with 100 mg of soy isoflavone per day for 12 weeks. The MRS response of the perimenopausal women showed a mean total score of 19.55% and a somatic subscale score of 27.7%. Among the postmenopausal women under evaluation, 12.62% showed improvement in their mean total scores, 14.54% in somatic subscale scores, and 26.79% in psychological subscale scores. These were improvements but possibly had little significance, from our point of view. However, through analysis of individual symptoms it was observed that the maximum improvement (40%) was seen in symptoms of hot flashes of mild to moderate degree in both perimenopausal and postmenopausal women. Severe to very severe hot flashes were seen only among perimenopausal women, which improved by 36.11% after treatment. Those are more relevant effects, but no identification or partial quantification of the various isoflavones in the supplement given to these women was performed. It is probable that this trial could not be reproduced.

7.6.2 Clinical Trials

In our search for "soy" and "isoflavones" we activated the following filters: "clinical trial," "free full text," "published in the last 5 years," and "humans." The same was

done with the clinical trials documented in the literature. Selected data are explained below.

In Italy, Bitto et al. (2010) performed a randomized, double-blind, placebo-controlled trial of therapy with 54 mg/day of genistein in 389 postmenopausal women with osteopenia for 24 months. A subcohort (138 patients) continued this therapy for an additional year. The trial was performed in Italy, but no details of the ethnicity of the subjects was mentioned. From the results, the investigators verified that the administration of the therapy for 3 years did not affect serum thyroid hormones or autoantibodies at the dose under evaluation. They also verified that in this clinical trial, the genistein aglycone positively regulated bone metabolism without harmful estrogenic activity. In fact, these results were theoretically expected, since, if we did a calculation of the TE, the affinity of genistein for ERα would be 1.044% and that for ERβ would be 23.709%, with a TERE calculation of about 1/4 relative to estrogen itself (Campos and Matos 2010). The main advantage was affinity for bones, which was due to the predominance in the affinity of genistein for ERβ. Nevertheless, the low affinity for ERα in this study corresponded to an affinity of 1.044%, which was considered nonestrogenic.

In China, Liu et al. (2012) evaluated the effect of dietary soy isoflavones on blood pressure with data collected from four selected trials. The duration of the intervention varied from 1 to 12 months. The sample sizes varied from 18 to 302 subjects, with an average age ranging from 48.5 to 66.7 years. The ingested soy protein ranged from 20 to 50 g/day and the isoflavone dosage from 65 to 153 mg/day. Briefly, no data on the ethnic groups included in the studies was mentioned. Additional subgroup analyses did not find any significant effects of gender, the duration of the intervention, or the doses of isoflavones or soy protein on SBP or DBP in response to soy isoflavone intake, but this intake did lead to a larger reduction in blood pressure in younger hypertensive subjects than in normotensive subjects.

In a meta-analysis of 11 trials, Liu et al. (2012) demonstrated that soy isoflavone intake resulted in mean decreases of 2.5 mmHg for SBP and 1.5 mmHg for DBP in comparison with placebo. Once again, they highlighted that the obvious heterogeneity detected among the 11 trials could primarily be ascribed to the blood pressure status of the subjects in the studies, and that the findings must be interpreted with caution in view of the limited statistical power.

More three clinical trials from the last 14 years are available, and the main conclusions are presented below.

In 2005, the Johns Hopkins University Clinical Trials Unit in the USA did a phase 4 clinical trial to evaluate the effects of an isoflavone-containing soy supplement called Revival (although there was no mention of the dose) on memory/cognition, quality of life, and hot flashes in men with prostate cancer undergoing testosterone suppression therapy (ClinicalTrials.gov study identifier: NCT00245518). The study sample consisted of 39 men aged 21 years or older with prostate cancer and hypogonadism for at least 3 months. The participants were given either a placebo or Revival once daily for 14 weeks. Blood was drawn to check their cholesterol levels and liver, kidney, thyroid, and prostate health. Urine was collected to check on bone markers. The patients completed questionnaires to test

their memory, attention span, and vocabulary. The hypothesis to be confirmed was that "treating men who have prostate cancer with daily Revival will result in at least a 50% reduction in hot flashes compared to placebo. Possible benefits may include increase in memory, decrease in hot flashes, and a general increase in quality of life." The results obtained were only from 33 participants who completed the study with no report of adverse events and were similar in some of the items except for hot flashes and sexual behavior. Full details can be obtained at https://clinicaltrials.gov/show/NCT00245518.

In 2017, Silva et al. published data from a prospective, randomized, double-blind trial in 30 postmenopausal women who were evaluated in the gynecology department at the Federal University of São Paulo (UNIFESP), Brazil. This was a phase 2 clinical trial to evaluate "postmenopausal facial skin after estradiol and genistein topical treatment." The aim of this trial was "to compare the effects of estradiol or genistein treatment on the hyaluronic acid concentration on the postmenopausal facial skin." The study subjects were postmenopausal women aged 45–55 years who met the study inclusion criteria of being 2–5 years postmenopause with a follicle-stimulating hormone (FSH) level >40 mU/mL, an estrogen level <20 pg/mL, and a body mass index <30 kg/m^2. They were divided into two groups: one treated with 17β-estradiol gel 0.01% ($n = 15$), and the other treated with genistein gel 4% (isoflavone; $n = 15$). The length of treatment was 24 consecutive weeks. Pre-auricular skin biopsies were performed on each patient before and after the treatment for evaluation of hyaluronic acid in the tissue. The materials were processed using immunohistochemical and biochemical methods (https://clinicaltrials.gov/show/NCT01553773). The study was completed, and the data showed an increase in the amounts of both type I and type III facial collagen by the end of both treatments. However, the outcomes of the estrogen-treated group were superior to those of the genistein-treated group, with statistical significance of $P < 0.001$. The authors concluded that "treatment with topical estrogen is superior to genistein, but both have positive impacts on facial skin collagen. Nevertheless, it is still unclear whether prolonged use of genistein and other topical phytoestrogens could produce systemic effects and further research is needed to clarify this question" (Silva et al. 2017). Caution is needed in the interpretation of this conclusion, in view of the fact that the incidence of skin cancers is increasing.

Menopause induces estrogen loss, which leads to increased bone loss. Sathyapalan et al. (2017) did a relevant study to help reduce osteoporosis postmenopause. Since soy isoflavones can act as selective estrogen receptor modulators, their role in bone turnover should be evaluated. The primary outcome was an assessment of changes in plasma bone turnover markers. The secondary outcomes were assessments of changes in cardiovascular risk markers, including insulin resistance, blood pressure, and the lipid profile. The trial was performed as a double-blind, randomized, parallel study in which 200 women were randomized within 2 years after the onset of menopause to receive 15 g of soy protein with 66 mg of isoflavone, or 15 g of soy protein alone, daily for 6 months. The type of isoflavone was not identified. From the results, the authors observed significant reductions in fasting glucose, insulin, and SBP, but there were no significant changes in the fasting lipid profile

or DBP with either preparation used in the study. There was a significant increase in thyroid-stimulating hormone (TSH) and a reduction in free thyroxine ($P < 0.01$). Even with a possible beneficial effect on bone health and an improvement in cardiovascular risk markers, the significant increase in TSH and the reduction in free thyroxine following soy supplementation indicated a detrimental effect on thyroid function. This is very important, as side effects should be addressed and caution is needed in the recommendation of these products, because sometimes the benefits are overestimated.

7.7 Safety: Toxicity and Side Effects

Most of the issues associated with safety, toxicity, and side effects have already been identified and discussed throughout this text. In this section, some other details are briefly discussed.

7.7.1 Infant Nutrition

Concerns have been raised over potential risks of soy protein formulas for infant nutrition – in particular, because of their isoflavone content. The authorities or pediatric societies in Australia, Canada, France, Ireland, New Zealand, Switzerland, and the UK have advised health professionals and caregivers that because of the limited availability of data, the use of soy protein formulas in infants should be restricted to specific cases. Following the publication of these guidelines, in 2006 the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGRAN) Committee on Nutrition summarized the available information on the composition and use of soy protein formulas as substitutes for breastfeeding and cows' milk protein formulas. The main concerns were safety and adequacy for the growth and development of infants. The committee evaluated soy as a source of protein that could be inferior to cows' milk, with lower digestibility and bioavailability, as well as having a lower content of methionine, which is the main product used in infant formulas for this purpose. They noted no nutritional advantage over cows' milk and discussed high concentrations of phytate, aluminum, and isoflavones, which could have untoward effects. Another important statement noted that there was no evidence supporting the use of soy protein formulas for the prevention or management of infantile colic, regurgitation, or prolonged crying (Agostoni et al. 2006). The conclusion emphasized that manufacturers should aim to reduce the concentrations of trypsin inhibitors, lectins, goitrogenic substances, phytate, aluminum, and phytoestrogens in soy protein formulas, but no specific information about isoflavones was provided.

Testa et al. (2018) did a review of studies published from January 1980 to June 2017 and made a statement about the use of soy-based formulas (SFs) in infant nutrition. They mentioned the belief that the use of SFs during infancy can reduce the risk of the development of several diseases later in life, but they did not find

definitive data that could substantiate these claims. With regard to risks, they noted the potentially toxic role of the phytoestrogens (isoflavones) contained in these products and pointed out that in vitro animal studies had yielded conflicting results and "raised suspicions that SF could have potentially negative effects on sexual development and reproductive function, neurobehavioral development, immune function, and thyroid function" (Testa et al. 2018). From their analysis of the literature, they concluded that "the impact of modern SFs on human development seems to suggest that their use is not associated with relevant abnormalities. Only children with congenital hypothyroidism need adequate monitoring of thyroid function." However, ethnic differences were not evaluated.

7.7.2 The Thyroid

For case reports in a PubMed search using the keywords "soy" and "isoflavones" with the filters "case reports," "free full text," "published in the last 10 years," and "humans" activated, only one item was found, but it is very relevant.

Nakamura et al. (2017) (from the Department of Internal Medicine at Saiyu Soka Hospital in Matsubara, Japan) reported a case involving soy isoflavones that induced hypothyroidism in a patient with chronic lymphocytic thyroiditis. Although the majority of the research data might lead one to suppose that the Asian population in general tolerates soybeans well, these authors emphasized that consuming health drinks that include soy isoflavone extracts can lead to severe hypothyroidism.

In various countries in North and South America, many people have thyroid pathologies such as hypothyroidism, and some other patients have potentially chronic lymphocytic thyroiditis. The causes are far from being identified, but some exogenous foods influence the thyroid. If foods interfere with the production of thyroid hormone, they can cause serious hypothyroidism, as could be the case with isoflavones from soy. This situation should be assessed very carefully now that food with soy is widespread all over the world.

Bitto et al. (2010) published data from 3 years of a study in 389 postmenopausal women taking 54 mg/day of genistein. The results showed that serum thyroid hormones and autoantibodies were not significantly influenced by the treatment. Despite this evidence, the safety of isoflavones still warrants further investigation, especially in ethnicities other than Asians.

7.8 Marketed Products

There is a compilation of the isoflavone content of foods provided by a study funded by the USDA and the US Army, which can be obtained from the website http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html (Project PHYTOHEALTH, grant agreement ID: QLK1-CT-2002-02453, 2003–2006).

The data presented in Campos and Costa (2012) show a wide range of isoflavone levels that different products in the market may provide to the consumer in

some European countries. Even when isoflavone extracts from soy are recommended as therapeutics in some over-the-counter (OTC) tablet preparations, the profile of the relative concentrations of the different constituents is not consistent across all brands (Campos et al. 2006). It is relevant to mention that the majority of soy suppliers breed a wide range of soy cultivars, and despite we showed in Figs. 2 and 3 similar HLPC profiles for isoflavones extracts, other cultivars present different relative concentrations of the main compounds that imply an unpredictable estrogen-like impact, as is explained above.

In the review published by Rizzo and Barini (2018), important data were given about the total grams of intake of soy protein or soy isoflavone and the relative variability of soy foods. They also included information about this intake in many countries around the world.

In recent decades, a new generation of soy products has come onto the market (e.g., yogurts, cheeses, soy milk drinks, and infant formulas) and commonly consumed food products now incorporate soy flour (e.g., bakery products) and protein isolates (e.g., meat products and soy meatless products such as soy burgers). In the last 15 years, the development of nutritional supplements rich in isoflavones has targeted the niche market of menopausal women. The EFSA (2012) report emphasized that "On the basis of the data presented, the Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of soy isoflavones and maintenance of bone mineral density, and between the consumption of soy isoflavones and reduction of vasomotor symptoms associated with menopause" and that "On the basis of the dietary intake estimates, for the high dietary intake adult and infant consumer, particularly where there may be indications of (congenital) hypothyroidism, large data gaps regarding critical windows of development, and timing of exposure remain outstanding and need to be addressed before any conclusive assessment on safety can be made."

7.9 Perspective/Conclusions

Even with such important data from many studies in different countries, the conclusions should be interpreted with caution. Observational studies among Asian populations have consistently found that the intake of isoflavone-containing whole soy and traditional soy-based foods can be related to a lower incidence of chronic disease or cancer. In contrast, studies performed in Western populations have frequently included controversial end points and failed to consistently demonstrate health benefits. Probably the main problem is the kind of products involved in the studies, as various combinations of soy and isoflavones are involved and no estrogenic-like activity of them has been evaluated in a way to give comparable results. Until now, the epidemiological and migratory evidence for the role of soy isoflavones in health prevention and promotion has been somewhat unclear because the ethnic groups under evaluation have not been specified in the equation. Current and ongoing clinical trials with soy may help us to further understand its role in breast and prostate cancer prevention, peri- and postmenopausal relief of specific

symptoms, and safe use in relation to the thyroid. Further studies in infant and adult humans are needed to address factors related to the observed effects of soy isoflavones in the global market, but the health impacts are diverse, depending on various factors (ethnicity, age, gender, etc.), and the risks associated with some of them could be pernicious.

7.10 Cross-References

► Antioxidants in Diets and Food
► Introduction of Phytonutrients

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Citrus Flavanones

8

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Contents

Abstract

Plant foods represent a very rich source of phytochemicals, including flavonoids that play a prominent role as healthy compounds. Flavonoids are object of numerous studies for their antioxidant, anti-inflammatory, neuroprotective, anti-cancer, anti-obesity, and antidiabetic activities.

Whereas some classes of flavonoids are distributed in a wide range of plants, others, such as flavanones, are found only in specific species. Indeed, flavanones are present almost exclusively in *Citrus* species (Rutaceae). The aim of this

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chapter is to report the most recent developments related to the bioactivities of *Citrus* flavanones, their bioavailability and metabolism, potential toxicity and side effects, application in foods, and critical analyses of their potential future application in food industries and for the formulation of pharmaceutical and nutraceutical products.

Keywords

Flavanones · Bioavailability · Bioactivities · Human studies · Application in food

8.1 Introduction

Plant foods represent a very rich source of bioactive compounds, among which are polyphenols. According to their chemical structures, polyphenols can be classified into phenolic acids, flavonoids, lignans, and stilbenes. The polyphenols that mainly characterize the human diet, representing about two-thirds and one-third of the total daily intake of these phytochemicals, are phenolic acids and flavonoids (Ovaskainen et al. 2008).

Several studies have reported an inverse relationship between flavonoids-rich foods and some degenerative diseases (Ma et al. 2018; Spagnuolo et al. 2015). According to their structures, dietary flavonoids are classified into six main groups, namely, anthocyanidins, flavanols, flavanones, flavones, flavonols, and isoflavones. Whereas some of these classes of flavonoids are distributed in a wide range of plant foods, others, such as flavanones, are found only in particular foods. In fact, this class of flavonoids is present almost exclusively in *Citrus* species. To a lesser extent, they are found also in some plants including tomatoes and mint. In *Citrus* fruits, flavanones account for approximately 95% of the total flavonoids (Peterson et al. 2006a, b). Flavanones occur mainly in flavedo, albedo, and segment membranes.

A total flavanones content in the range of 35–147 mg/100 g was found in *C. sinensis* (orange) (Peterson et al. 2006b). Naringin and narirutin were quantified in *C. paradise*. A content in the range of 44–106 mg/100 g was demonstrated (Peterson et al. 2006b). However, because generally in the preparation of juices, the albedo and membranous parts are discarded, flavanone levels in *Citrus* juices are lower. Indeed, levels of naringenin in the range of 17–76 mg/100 mL were found in the juice of *C. paradisi* (Ross et al. 2000) and levels of hesperidin and narirutin in the range 13–77 mg/100 mg were found in the juice of *C. sinensis* (Tomas-Barberan and Clifford 2000).

In Europe, *C. sinensis* represents the major source of *Citrus* flavanones (Zamora-Ros et al. 2010). Flavanones are present in all cultivars. However, red cultivars showed a higher number of flavanones (Grosso et al. 2013). *Citrus* fruits are largely consumed as fresh or as juices or are canned. Moreover, uses in cosmetic and pharmaceutical industries are reported. *Citrus* fruits have been used in traditional medicinal medicines of several countries including China, Korea, and Japan. In particular, Chinese Pharmacopoeia reports six *Citrus* species, namely, *C. aurantium* L., *C. medica* L., *C. sinensis* Osbeck, *C. reticulata* Blanco, and *C. wilsonii* Tanaka (Lv et al. 2015) to treat skin

inflammation, indigestion, cough, ringworm infections, muscle pain, and to lower blood pressure. Both in vitro works and evidences from epidemiological, clinical, and preclinical studies described the healthy promoting properties of *Citrus* species and flavanones (Kang et al. 2016; Loizzo et al. 2012, 2016; Rendeiro et al. 2016; Tundis et al. 2012; Zaidun et al. 2018; Zhou et al. 2018). The purpose of this chapter is to compile data related to the bioactivities of *Citrus* flavanones, their bioavailability and metabolism, application in foods, marketed products, potential toxicity and side effects, and critically analyze the future perspectives.

8.2 Bioactive Constituents of *Citrus* Species

In the *Citrus* genus, different types of flavonoids have been identified: flavanones, flavones, flavonols, and – uniquely to blood oranges – anthocyanins. The flavonoids contained in greater quantities in *Citrus* fruits are flavanones, representing approximately 95% of the total (Peterson et al. 2006b). This subclass of polyphenols is the intermediate structure for the biosynthesis of a wide range of flavonoids found throughout the plant kingdom. In the vegetal tissues, flavanones result from the condensation of two precursors: three molecules of malonyl-CoA with one molecule of *p*-coumaroyl-CoA by chalcone synthase (CHS), forming the 2′,4′,6′,4-tetrahydroxychalcone or naringenin chalcone (Fig. 1) (Forkmann and Heller 1999).

Eventually, chalcone isomerizes into (2S)-flavanone (5,7,4′-trihydroxyflavanone), otherwise known as naringenin. This reaction can occur spontaneously, due the instability of the chalcone structure or stereospecifically by chalcone–flavanone isomerase (CHI), which plays a significant role in the cyclization reaction of chalcones to form all flavonoid classes (Forkmann and Heller 1999; Aoki et al. 2000). The main *Citrus* flavanones aglycones are naringenin (5,7,4′-trihydroxyflavanone), hesperetin (4′-methoxy-3′,5,7-trihydroxyflavanone), eriodictyol (5,7,3′,4′-tetrahydroxyflavanone), and isosakuranetin (4′-methoxy-5,7-dihydroxyflavanone) (Fig. 2).

In *Citrus* fruits, these four compounds are generally glycosylated at position 7 by either the disaccharide neohesperidose or rutinose (Fig. 3) producing four neohesperidose glycosides, namely, naringin, neoeriocitrin, neohesperidin, and poncirin, and four rutinose glycosides, namely, didymin, eriocitrin, hesperidin, and narirutin (Peterson et al. 2006b).

Neohesperidosides derivatives have a bitter taste, while rutinosides derivatives have no taste. Surprisingly, chemical hydrogenation of neohesperidin in alkaline solution produces the semisynthetic neohesperidin dihydrocalcone, a potent artificial sweetener (Kinghorn et al. 2010).

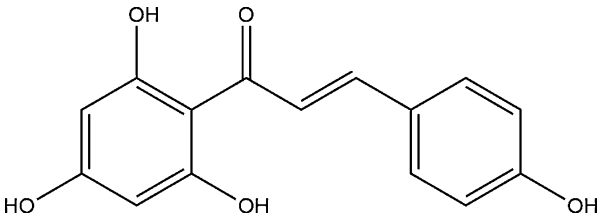

Fig. 1 Naringenin chalcone

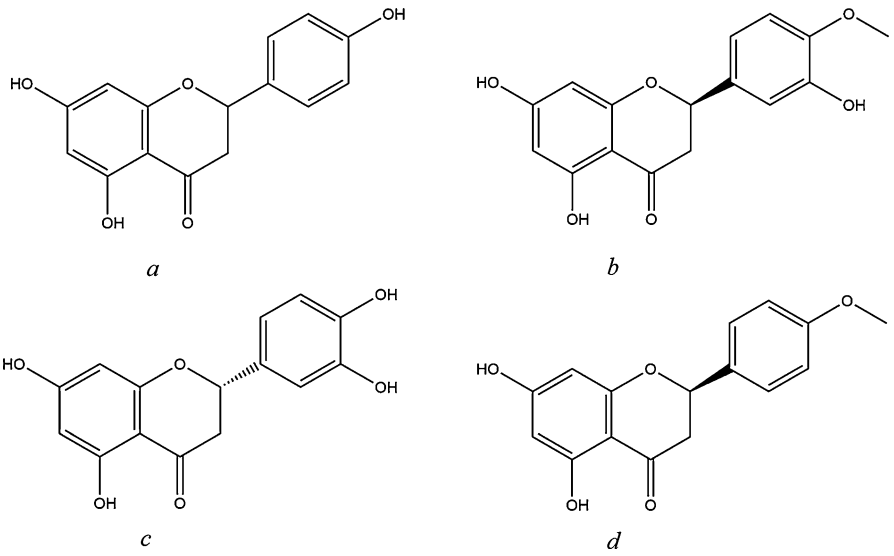

Fig. 2 *Citrus* flavanone aglycones: *a* naringenin, *b* hesperetin, *c* eriodictyol, *d* isosakuranetin

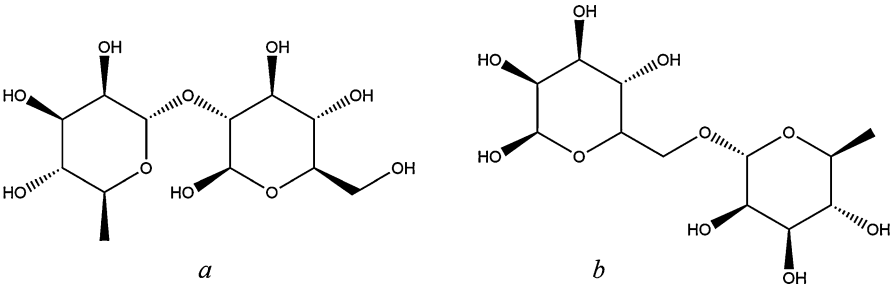

Fig. 3 Sugars present in *Citrus* flavanones glycosides: a neohesperidose and b rutinose

8.3 Bioavailability and Metabolism of Flavanones

To exert their beneficial properties, polyphenols should be released during digestion and absorbed (Parada and Aguilera 2007). Several works investigated how much of polyphenols can be absorbed after ingestion. Nevertheless, the poor bioavailability of these molecules have been highlighted.

Generally, the bioavailability of polyphenols is dependent on different factors including bioaccessibility, transport, and metabolism (Bouayed et al. 2012). Most studies that analyzed the bioavailability of *Citrus* flavanones involved hesperetin, naringenin, and their glycosides. After intake, flavanone glycosides are hydrolyzed in the small intestine and colon.

Subsequently, during the passage across small intestine and liver, aglycones are converted into their respective metabolites such as glucuronides, sulfoglucuronides, and sulfates, which are distributed at the various cell sites. A considerable quantity can be found in urine (Matsumoto et al. 2004). The deglycosylation of naringenin-7-*O*-β-D-glucoside happens early in the small intestine.

Naringenin-7-*O*-β-D-rhamnoglucosides occur in the colon (Choudhury et al. 1999). Indeed, Felgines et al. (2000) showed the presence of naringenin conjugates within 3 h in the plasma of rats fed with naringenin or its 7-*O*-β-D-glucoside. No naringenin metabolites neither naringenin-7-*O*-β-D-rhamnoglucoside were found. However, in the same work, similar naringenin concentrations after 10 h of ingestion were found regardless of the diet. This evidence demonstrated the delayed intestinal absorption of naringenin rhamnoglucosides.

Naringenin and its glucuronides were found in plasma and brain of rats 10 min after administration of a concentration of 20 mg/kg (Peng et al. 1998). Higher concentrations of naringin were detected in the bile and liver (Tsai 2002). Manach et al. (2003) confirmed that in humans, both naringin and hesperidin are absorbed in the distal part of the intestine, and once deglycosylated, during their transfer from the luminal side of the gut to the portal vein, aglycones are sulfated and/or glucuronated by uridine diphosphate (UDP)-glucuronosyltransferase and sulfotransferase.

Erlund et al. (2001) evaluated the concentration in human plasma of naringenin and hesperetin aglycones after ingestion of grapefruit or orange juice (8 ml/kg body weight). A range of 0.6–6.0 mmol/l with a peak concentration of 6.0 mmol/l for naringenin (from grapefruit juice) and 2.2 mmol/l for hesperetin (from orange juice) was found. A considerable distribution to tissues for both flavanones was suggested (Erlund et al. 2001). Successively, the ingestion of 135 mg of both hesperetin and naringenin under fasting conditions in 6 volunteers resulted in their appearance as metabolites in blood plasma 20 min later. Peak plasma concentration of 2.7 and 7.4 mmol/l for hesperetin and naringenin was reached 4.0 and 3.5 h after ingestion, respectively (Kanaze et al. 2007).

After ingestion of 1 l of orange juice (characterized by a content of 444 mg of hesperidin and 96.4 mg of narirutin), analysis over 24 h led to C(max) values at 1.28 and 0.20 mmol/L for hesperetin and naringenin (after deconjugation), respectively. The levels in urine of flavanones, expressed as percentage of their intake, amounted to 7.9% and 6.4% for naringenin and hesperetin, respectively. The dose not considerably affected the urinary excretion of flavanones (Manach et al. 2003). Generally, naringenin is more bioavailable than hesperetin as resulted by both plasma and urine analyses (Gardana et al. 2007; Kanaze et al. 2007).

The study of Cao et al. (2010) aimed to explain the different efficacy of two Zhi Zhu Wan (ZZW) varieties on the basis on the results of pharmacokinetics of hesperetin and naringenin. ZZW is a Chinese formulation that contains *Atractylodes Rhizome* and *Fructus Citrus Immaturus*, which derived from *C. aurantium* and *C. sinensis*. Although the immature *C. aurantium* and *C. sinensis* fruits showed many common constituents, there are significant differences in their quantity and in the presence of other characteristics compounds (Liu et al. 2008; Wang et al. 2008). These chemical differences reflect the different clinical uses. After oral

administration of *C. aurantium*, both naringenin and hesperetin were detected in plasma and demonstrated similar pharmacokinetic parameters. After oral administration of *C. aurantium*, both hesperetin and naringenin were detected in plasma and showed comparable pharmacokinetic parameters. After oral administration of *C. sinensis*, only hesperetin was detected. Moreover, it was found that the pharmacokinetic properties for hesperetin in *C. sinensis* was different from hesperetin in *C. aurantium*, and it was slowly eliminated. Based on all these results, it can be concluded that hesperetin can be considered the effective compound for both ZZW varieties.

The main plasma and urinary metabolites of aglycones are obtained by glucuronidation and sulfation pathways. Glucuronide metabolites dominate with a percentage of about 87%. However, the importance of the other metabolites should not be neglected (Manach et al. 2003). Hesperetin-7-*O*-β-D-glucuronide, hesperetin-3′-*O*-β-D-glucuronide, hesperetin sulfoglucuronide, and hesperetin diglucuronide are the main metabolites of hesperetin (Matsumoto et al. 2004; Mullen et al. 2008). Naringenin-7-*O*-β-D-glucuronide, naringenin-4′-*O*-β-D-glucuronide, naringenin-4′-*O*-sulfate-7-*O*-β-D-glucuronide, naringenin-4′,7-*O*-disulfate, and naringenin-4′-*O*-β-D-glucuronide-7-*O*-sulfate are the most abundant metabolites of naringenin (Brett et al. 2009; Tripoli et al. 2007).

A good determinant of flavanones intestinal absorption is their ability to permeate of epithelial cells. The faster absorption of aglycones in comparison to glycosides was demonstrated in humans by Miyake et al. (2006) that investigated the mechanism of absorption of eriocitrin and eriodictyol.

The transport of flavonoids across the cell membranes in general imply the presence of ATP-binding cassette (ABC) transporters, present in the enterocytes apical or basolateral membrane, and allow the excretion back into the intestinal lumen or uptake into the blood, respectively. Intestinal ABC transporters related to the flavonoids include breast cancer resistance protein (BCRP), P-glycoprotein, and multidrug resistance proteins. Kobayashi and Konishi (2008) confirmed that the aglycone hesperetin was better absorbed across Caco-2 cell monolayers in comparison to its glycoside hesperidin. The absorption occurs through transcellular passive diffusion and a proton-coupled active transport (Kobayashi et al. 2008). In the same year, Brand et al. (2008) revealed that hesperetin-7-*O*-β-D-glucuronide and hesperetin-7-*O*-sulfate are mainly transported to the apical side in Caco-2 monolayers. Instead, it was demonstrated that hesperetin aglycone is able to permeate the basolateral side of the monolayers of Caco-2 cell line. The results from this work showed that BCRP-mediated transport could be one of the key steps that could limit the bioavailability of hesperetin (Brand et al. 2008). Successively, Brand et al. (2010) analyzed the differences in metabolism and transport, and the activity of separated hesperetin enantiomers in in vitro models by using (i) human intestinal fractions that contain UDP-glucuronosyl transferases or sulfotransferases, (ii) Caco-2 cell monolayers, and (iii) mouse Hepa-1c1c7 cells transfected with human EpRE-controlled luciferase.

These results showed relatively small differences in the metabolism, transport, and activity between (S)- and (R)-hesperetin. This is important for the consideration

that experiments that are performed with commercially available racemic hesperetin may adequately reflect what can be expected for the naturally occurring (S)-hesperetin.

Taking onto account that the bioavailability of healthy compounds may be influenced by the food matrix in which they are to be found, the bioavailability and metabolism of *C. sinensis* juice flavanones, namely, naringenin-7-*O*-rutinoside and hesperetin-7-*O*-rutinoside, and the impact of the ingestion of the juice with a yogurt were studied (Mullen et al. 2008). Human plasma and urine were collected over a 24 h after the consumption of juice (250 mL) with and without of yogurt (150 mL). Juice contains 168 and 12 μmol of hesperetin-7-*O*-rutinoside and naringenin-7-*O*-rutinoside, respectively, paracetamol (1 g) and lactulose (5 g). It was demonstrated that the investigated dairy food matrix may delay the intestinal absorption of both naringenin-7-*O*-rutinoside and hesperetin-7-*O*-rutinoside with no impact on their bioavailability. The role of the dietary matrix (yogurt) on the bioavailability of flavanones was studied in a successive work in which it was demonstrated that the quantity of flavanone metabolites excreted in urine after 24 h of *C. sinensis* juice consumption has reduced about 7 times when the orange juice is ingested with yoghurt (Roowi et al. 2009). Probably, this reduction is due to the alteration of flavanones metabolism by the action of the microflora in the large intestine.

Flavanones demonstrated to be resistant to oxidative reactions, while their isomerization into chalcones was observed mainly for the orange juice. The processing of pasteurization for 30 s at 95 °C, freezing, and concentration of juice showed no effect on the in vitro flavanones bioaccessibility (Gil-Izquierdo et al. 2002). Chalcones were found in higher quantities in industrially pressed juice in comparison to the manually pressed juice.

Spigoni et al. (2017) recently investigated the bioavailability in humans of different metabolites of *Citrus bergamia* (bergamot) phenolics (including flavanones) after juice intake. In particular, the potential use of bergamot in the system of lipotoxicity-induced myeloid angiogenic cells (MACs) impairment was analyzed. MACs play a crucial role in endothelial functionality and repairing processes. However, the lipotoxic effects of some compounds, such as palmitic acid and stearic acid, may decrease these activities. After consumption of bergamot juice, the circulating flavanone metabolites in plasma and urine were assessed. Twelve flavanone phase II conjugates were identified and quantified. Then, the effects at physiological concentrations of hesperetin-7-*O*-glucuronide, naringenin-7-*O*-glucuronide, hesperetin-3′-*O*-glucuronide, and naringenin-4′-*O*-glucuronide were investigated on gene expression of apoptosis and inflammation markers in MACs after exposition to stearic acid. Hesperetin-7-*O*-glucuronide and naringenin-4′-*O*-glucuronide demonstrated to be able to mitigate stearate-induced inflammation in MACs.

Previously, Aschoff et al. (2016) analyzed the bioavailability and the colonic catabolism of flavanones from orange juice by using a dose 2.4-fold higher in comparison to the flavanones from fruits. This choice is due to the consideration that the juice contains flavanones at lower concentrations as compared to the fruits. In this randomized two-way cross-over project, twelve healthy subjects are invited to

consume a meal including either pasteurized juice or fresh fruits, providing 751 and 1774 μmol as total *Citrus* flavanones content, respectively.

Naringenin, deglucuronidated and desulfated hesperetin, and four catabolites, namely, hippuric acid, 4-hydroxyhippuric acid, 3-(3′-hydroxy-4′-methoxyphenyl) propionic acid, and 3-(3′-hydroxyphenyl)hydracrylic acid, were quantitated in 24-h urine.

The urinary excretion of hesperetin as well as postprandial catabolites excretion showed nonsignificant differences after consumption of both orange juice and fruits. In conclusion, the excretion of flavanones after ingestion of orange fruits did not differ from that succeeding at juice consumption, although the use of a 2.4-fold higher dose. Probably, this is due to a saturation of absorption or flavanones entrapment in the matrix of the fruit that is rich in fibers.

At the end of this paragraph, some general considerations are necessary. Numerous studies show marked individual differences in the bioavailability of this class of compounds, due to both molecular factors, including the activity and/or synthesis of enzymes responsible of the biotransformation and transporters, and physiological factors, including gastric motility, body composition, and body weight. Variations have been described for biotransformation enzymes and secretory transporters associated with flavonoids, including uridine diphosphate glucuronosyltransferases, CYP3A4, and P-glycoprotein (Dai et al. 2001; Fisher et al. 2000; Hall et al. 1999; Lown et al. 1995; van der Kolk et al. 2000). Determinant for the bioavailability of flavonoids are also the composition and activity of the gastrointestinal microflora.

8.4 Bioactivities of *Citrus* Flavanones: *Animal Experiments*

Flavanones extracted from *Citrus* fruits are involved in many essential physiological functions: as an antioxidant, anti-inflammatory, and antitumor activity (Table 1). They are able to counteract the endogenous and exogenous biological stimuli. They can also reduce cholesterol and triglyceride levels in experimental animals (Jeon et al. 2007; Habauzit et al. 2011; Horcajada et al. 2008).

Citrus flavanones also possess antioxidant activities, although these activities are poorer in respect to many other polyphenols (Jeon et al. 2002). Their anti-inflammatory, antitumor, anti-atherogenic properties cannot be explained solely based on their antioxidant properties. Investigations of their mechanism of action suggested that they can act as free radical scavengers and, also, modulate cellular signalling processes or may themselves serve as signalling molecules.

Several animal studies have been published during the past few years (Acquaviva and Iauk 2010; Nijveldt et al. 2001; Ross and Kasum 2012). A lot of attention has been paid to the anticarcinogenic properties of flavanones. In particular, hesperidin has been shown to inhibit mammary, urinary bladder, and colon carcinogenesis in laboratory animals (So et al. 1996; Yang et al. 1997; Tanaka et al. 1997; Miyagi et al. 2000).

Table 1 Major effects of flavanones

Flavanones	Effects	Mechanisms of action	References
Hesperidin Naringenin Naringenin	Antioxidant	ROS ⇓ Antioxidant enzymes ⇑ Lipid peroxidation production ⇓ Nonenzymic antioxidants ⇑	Acquaviva and Iauk 2010 Akiyama et al. 2010 Nandakumar and Balasubramanian 2011
Hesperidin Naringenin Naringenin	Anticancer	Apoptosis ⇑ DNA binding of NFkB ⇓ DNA binding of tumor initiation ⇓ iNOS ⇓ COX-2 ⇓ Regulation of both phase I and phase II metabolizing enzymes	Acquaviva and Iauk 2010 Leonardi et al. 2010 Nandakumar and Balasubramanian 2011
Hesperidin Naringenin Naringenin	Anti-lipidemic	Cholesterol ester synthesis ⇓ 3-Hydroxy-3-methylglutaryl-coenzyme A reductase ⇓ Acyl coenzyme A: cholesterol O-acyltransferase ⇓ LDL⇓ Cholesterol levels⇓	Lee et al. 1999a,b Kurowska et al. 2000
Hesperidin Naringenin Naringenin	Antidiabetic	ALT⇓ AST⇓ GLUT-4 antagonism Modification of insulin sensitivity Improve glycolytic and gluconeogenetic enzymes Impeded TNF-α expression Induces AMPK	Akiyama et al. 2010 Priscilla et al. 2015 Vinayagam and Xu 2015
Hesperidin Naringenin Naringenin	Anti-neurodegenerative	ROS ⇓ Antioxidant enzymes ⇑ Counteract the degeneration of the nigrostriatal dopaminergic projection Anti-inflammatory activity ⇑ TNF-α ⇓ Aβ deposition ⇓ APP expression⇓	Leem et al. 2014 Li et al. 2015

Flavanones may act with different mechanisms of action, such as inhibiting carcinogen activation, stimulating carcinogen detoxification, scavenging free radical species, controlling cell cycles, inhibiting cell proliferation, inducing cellular apoptosis, and inhibiting angiogenesis, metastasis, and growth factors activity.

Nandakumar and Balasubramanian (2011) have investigated the antigenotoxic activity of hesperidin and have demonstrated that the daily administration of this compounds (30 mg/kg BW) for 45 days prevented 7,12-dimethylbenz(α)anthracene (DMBA)-induced experimental breast cancer formation. Probably this effect is due to the regulation of both phase I and phase II metabolizing enzymes and due to its strong antioxidant activity.

In addition, in vivo studies have shown that naringenin could suppress the early stage of colon cancer by attenuating inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) levels in carcinogen-injected rats (Leonardi et al. 2010). It is known that inducible iNOS is another enzyme that plays a pivotal role in mediating inflammation. Therefore, the anti-inflammatory effect of flavanones may be due both to their antioxidant activity and to their effect on enzymes involved in the inflammatory cascade.

It was demonstrated that phenolic compounds present in the juice of blood oranges might prevent lipid peroxidation and the formation of atherosclerotic plaques (Acquaviva and Iauk 2010; Sorrenti et al. 2004). Hesperidin and naringenin also act on lipid metabolism; in fact, they are able to regulate the apolipoprotein B secretion by HepG2 cells, probably through the inhibition of cholesterol ester synthesis, and to inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase and acyl coenzyme A cholesterol O-acyltransferase in rats (Lee et al. 1999a, b).

Moreover, a decrease in serum LDL and hepatic cholesterol levels was observed in rabbits fed with a cholesterol-rich diet and supplemented with orange juice (Kurowska et al. 2000).

An increase in high-density lipoprotein levels has been shown in hypercholesterolemic patients who consumed orange juice (Erlund 2004). Other biological activities attributed to naringenin and hesperidin include antidiabetic properties.

It is known that peroxisome proliferator-activated receptor gamma (PPARγ) plays a critical role in peripheral glucose homeostasis and in modulating insulin sensitivity. PPARγ induces glucose uptake by directly or indirectly increasing the transcription of genes encoding proteins such as GLUT4. The partial antagonism, exerted by flavanones, may be useful in improving insulin sensitivity (Vinayagam and Xu 2015). Recently, it was demonstrated that a 4-week diet-based administration of hesperidin (10 g/kg), in streptozotocin (STZ)-induced diabetic type 1 rats, induced a decrease of blood glucose compared to nondiabetic rats. In addition, hesperidin did not influence both bone metabolism and body weight in diabetic rats. Moreover, the antidiabetic effect of hesperidin may be due to its ability to inhibit pancreatic damage, thanks to its antioxidant activity, increasing insulin secretion and consequently decreasing glucose levels, and altering glycogen contents in the diabetic tissues by the improvement of glycolytic and gluconeogenetic enzymes (Akiyama et al. 2010). In addition, several studies showed that also naringenin possesses an insulin-mimetic effect; naringenin, in fact, is able to decrease blood glucose levels in healthy male Wistar rats (Vinayagam and Xu 2015). In particular, the oral administration of naringenin (25 mg/kg bw) for 45 days exerts a significant inhibition of intestinal α-glucosidase activity in diabetic rats induced by streptozotocin (STZ). This effect delays the absorption of carbohydrates with a consequent significant decrease in postprandial glycemic levels. Moreover, naringenin treatment, in diabetic rats, improved GLUT-4 and induced 5′ adenosine monophosphate-activated protein kinase (AMPK) activation, that increases glucose tolerance and insulin sensitivity, while at the same time decreasing TNF-α expression and ALT, AST levels in the serum, preventing STZ-induced liver damage in rats (Priscilla et al. 2014, 2015).

Scientific research has shown that flavanones can provide benefits in the prevention and treatment of metabolic diseases due to their antioxidant activity and their ability to modulate the expression of some proteins involved in hyperglycemia and hyperlipidemia.

It is reported that flavanones possess neuroprotective effects against various types of insult associated with neurodegenerative diseases, including Parkinson and Alzheimer diseases.

The neuroprotective activity of the *Citrus* flavanones may be due to their capacity to cross the blood-brain barrier and to arrest free radical-induced oxidative damage, which is known to play a pivotal role in many degenerative diseases. Antunes et al. (2014) have demonstrated that hesperidin mitigated both the increased levels of ROS and the activity of glutathione reductase induced by 6-hydroxydopamine.

Moreover, their neuroprotective action is mediated by the interaction with specific intracellular targets that are implicated in several signalling pathways important for maintaining the cellular homeostasis. Naringin is able to counteract the degeneration of the nigrostriatal dopaminergic (DA) projection by increasing the level of glia-derived neurotrophic factor (GDNF) in nigral DA neurons, with a concomitant activation of mammalian target of rapamycin complex 1 (mTORC1) (Leem et al. 2014). Moreover, naringin exerts its anti-inflammatory activity in CNS mitigating the increase of TNF-α induced by 1-methyl-4-phenylpyridinium (MPP+) in microglia (Leem et al. 2014).

Li et al. (2015) demonstrated that the pre-treatment with hesperidin (100 mg/kg body weight) for 10 days recovered deficits in non-cognitive nesting capability and social interaction and attenuated Aβ deposition, plaque-associated amyloid precursor protein (APP) expression, and microglial activation and TGF-β1 immuno-reactivity in both cerebral cortex and hippocampus of transgenic APP/PS1 APP/PS1 mice. Certainly further clinical studies must be performed to validate the neuroprotective activities of the flavanones.

8.5 Benefits (Human Studies)

Citrus flavanones have shown many interesting effects in vivo and in vitro models, including antioxidant properties, cholesterol and triglycerides reduction, cell proliferation and angiogenesis inhibition, estrogenic activity, nitric oxide (NO) level modulation, and decrease of lymphocyte immobilization and platelet aggregation (Bellocco et al. 2009; Barreca et al. 2009; Codoner-Franch and Valls-Belles 2010). Instead, their effectiveness in humans remains controversial because of both the intersubject variability in flavanones pharmacokinetics and the chemical transformation carried out by gut microbiota that in turn influences their absorption and biological activity as well.

Citrus healthy properties are mostly related to their antioxidant capacity. However, in healthy subjects, *Citrus* flavanones do not have significant antioxidant effects, suggesting that their antioxidant potency is negligible in normal conditions (Testai and Calderone 2017). Conversely, they can improve endogenous antioxidant

defense in non-healthy individuals, allowing a reduced risk of certain chronic diseases and the prevention of some cardiovascular disorders as well as certain kinds of cancer (Tomás-Navarro et al. 2014).

Rangel-Huerta et al. (2017) demonstrated that the consumption of an orange juice with a high content of flavanones improves oxidative stress and inflammatory biomarkers by decreasing the serum levels of hydroxyoctadecadienoic acid (9-HODE+13-HODE) and dihydroxyoctadecanoic acid (12,13-DiHOME and 9,10-DiHOME), as well as increasing the levels of 12-hydroxyeicosatetraenoic acid (12-HETE) compared to normal flavanones juice in 30 subjects from the BIONAOS study (Biomarkers In Overweight And Obese Adults).

Moreover, *Citrus* flavanones also have anti-inflammatory properties, mainly hesperetin, naringenin, and their glycosylated derivatives (Barreca et al. 2017). These molecules are capable of regulating cellular inflammation process, due to their antioxidant properties, but also by interacting with key enzymes (such as protein kinase, phosphodiesterase, lipoxygenase, cyclooxygenase, and phospholipase), inhibiting arachidonic synthesis, downregulating of NFkB activation and consequently of pro-inflammatory cytokines (TNF-a, IL-6, and IL-1), chemokines, COX-2, and iNOS (Tomás-Navarro et al. 2014; Bodduluru et al. 2016). Results of in vivo research and clinical trials support their use as compounds readily available at a low cost without any side effects or intolerance.

8.5.1 Cardiovascular Disease and *Citrus* Flavanones

Cardiovascular diseases (CVD), including myocardial infarction, coronary heart disease, strokes, cardiomyopathy, and other heart disease, are one of the leading causes of morbidity and mortality in worldwide. Epidemiological, clinical, and preclinical studies have deeply analyzed the relationship between *Citrus* flavanone intake and the risk of cardiovascular disease (Hollman et al. 2010; Testai and Calderone 2017). Their results suggest that *Citrus* flavanones prevent cardiovascular disease positively influencing some cardio-metabolic parameters (He et al. 2006; He et al. 2007; Gan et al. 2015) that are the main risk factors of CVD, such as hypertension, dyslipidemia, overweight, and hyperglycemia.

A number of sources suggest the cardioprotective activity of hesperidin and its aglycone form, hesperetin in particular (Barreca et al. 2017). A prospective study carried out on approximately 10,000 Finnish men and women reveals a 20% reduction in cerebrovascular diseases in those who consumed the highest levels of flavanones (4.7–26.8 mg aglycone/day) (Knekt et al. 2002).

A significant inverse correlation between flavanone consumption and cerebral ischemia has been found in women who consume high levels of flavanones (>63 mg/day) *versus* low levels (<13.7 mg/day) (Cassidy et al. 2012). Moreover, a systematic review and meta-analysis of prospective cohort studies reported that flavonoids intake, especially of flavanones, was associated with a decreased risk of cardiovascular disease ($p = 0.002$) (Wang et al. 2014).

Many clinical trials have focused their attention on modulation of CDVs risk factor by *Citrus*.

A meta-analysis of three randomized clinical trials, including 233 patients, demonstrated a correlation between grapefruit intake and a reduction in systolic blood pressure and waist circumference in overweight and obese adults (Onakpoya et al. 2017). In general, these effects of flavanones on hypertension, particularly naringenin and hesperetin, are due to improvement of vasodilation and endothelial function (Salehi et al. 2019) through endothelial production of NO and activation of voltage-operated calcium channels and potassium currents (Liu et al. 2014).

Metabolic syndrome, characterized by altered glucose metabolism, elevated blood pressure, dyslipidemia, and obesity is one of the main cardiovascular risk factor. A cohort clinical trial performed on 10,000 Polish subjects reveals that habitual consumption of flavonoids, among which flavanones, reduces the incidence of metabolic syndrome (Grosso et al. 2017).

Rizza et al. (2011) carried out a clinical trial of 3 weeks on 28 subjects with metabolic syndrome demonstrating that hesperidin (500 mg/day) reduces E-selectin expression, cholesterol, and ApoB level and induces enhancement of NO levels.

Intake of 300 mL of fruit juice (containing 95% of *Citrus* flavonoids) produces no variations of glucidic parameters but improvement of lipidic panel, with decrease in the cholesterol, LDL-C and C-reactive peptide levels, in subjects with metabolic syndrome ($n = 33$) compared with healthy subjects ($n = 20$) in a clinical trial of 4 or 6 months (Mulero et al. 2012).

High level of lipoprotein and cholesterol in plasma as well as hyperinsulinemia and hyperlipidemia led to development of atherosclerosis with the plaque formation in arteries, which is a gateway for cardiovascular diseases. Among flavanones, naringenin is the most effective to decrease triglycerides and LDL-C and inhibit glucose uptake as well. On the contrary, it promotes the improvement of high-density lipoprotein (HDL-C) level and antioxidant defenses, decreasing atherosclerosis-related genes expression (Orhan et al. 2015). These effects can be due to molecular structures characterized by the presence of a hydroxyl mevalonate moiety resembling that of statins, a drug able to inhibit cholesterol biosynthesis (Barreca et al. 2017).

Clinical evidences show that 400 mg/day of naringenin administrated for 2 months causes a reduction of the LDL-C, cholesterol and ApoB levels and an increase in HDL-C levels and detoxifying enzyme only in hypercholesterolemic subjects ($n = 30$) (Jung et al. 2003).

In addition, bergamot fruit shows anti-cholesterolemic properties both in animal and human studies (Cappello et al. 2016; Constans et al. 2015). A *C. bergamia* extract (Bergavit® containing 150 mg of flavonoids with 16% neoeriocitrin, 47% neohesperidin and 37% naringin) reduced cholesterol levels and improved lipidic and lipoproteic panel in mild hypercholesterolemic patients ($n = 88$) (Toth et al. 2016).

The results from clinical trial are controversial. In fact, Morand et al. (2011) observed no difference in fasting glucose, insulinemia, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in 24 overweight subjects after

drinking juice contained 292 mg of hesperidin and 47.5 mg of narirutin for 4 weeks. This flavanone combination, instead, reduced pressure parameter (4 mmHg), ameliorated postprandial microvascular reactivity, and some biomarkers of oxidative stress. Similar results have been obtained with a combination of naringin and hesperidin (500 mg plus 800 mg) in a randomized controlled study that saw 4 weeks of treatment involving moderately hypercholesterolemic patients. These findings, according to some authors, could be related to the difference in individual pharmacokinetic parameters.

Several clinical trials have demonstrated the positive effect of *Citrus* flavonoids in the reduction of cardiovascular risk factors. Although some of the mechanisms responsible for the beneficial effects of *Citrus* flavanones on the cardiovascular system are unclear, the nutraceutical value of these fruits in cardiovascular disease therapy should be considered as valid approach.

8.5.2 Diabetes and *Citrus* Flavanones

Type 2 diabetes mellitus (T2DM) represents a worldwide health problem and is a chronic, progressive disease characterized by elevated blood glucose levels and insulin resistance.

Chronic hyperglycemia causes complications, such as cardiovascular disease, diabetic retinopathy, renal dysfunction, and leg ulcers (Marathe et al. 2017). A healthier diet plays a key role in the T2DM prevention as suggested by various epidemiological studies (Schwingshackl et al. 2017). Nutraceutical products from plants have been reported to reduce hyperglycemia and lipid disorders in individuals with T2DM or with a predisposition to T2DM (Kaleem and Ahmad 2018).

Among *Citrus* flavanones, naringin and naringenin have been shown to control diabetes and its related complications in animal and cell studies by improving glucose tolerance and insulin sensitivity but also reducing plasma and/or hepatic cholesterol and triglyceride levels (Assini et al. 2013; Sharma et al. 2015).

A nutraceutical product rich in *Citrus* flavanones, limonoids, and tocotrienols (Diabetinol®) showed a significantly reduced peak hyperglycemic response, TC, and LDL-C levels and decreased HbA1c levels after 3 months supplementation in subjects with mild impaired fasting glucose (Judy et al. 2010). Similarly results have been achieved in subjects with greater severity of impaired fasting glucose ($\leq$15.4 mmol/L) in a 24-week, randomized, double-blind, placebo-controlled, parallel study carried out by Evans (Evans et al. 2015).

Ethanol extracts of *C. junos* (yuja) peel (YE) contain naringin, hesperidin, rutin, quercetin, and tangeretin, showing antidiabetic properties in animal models (Kim et al. 2013), and have been assayed by individuals with impaired fasting glucose (IFG) in an 8-week, randomized, double-blinded, crossover, placebo-controlled clinical trial. The authors reported that YE significantly reduced fasting plasma glucose levels, fasting plasma insulin, postprandial glucose, and c-peptide and homeostatic model assessment-insulin resistance compared to the placebo group (Hwang et al. 2015).

A body of evidence suggests that oxidative stress is a main mechanism in pathogenesis of diabetes. Bonina et al. (2002) evaluated some serum oxidative stress biomarkers in T2DM patients ($n = 33$) who took ROC supplement (50 mg/d, orally) for 2 months. They observed an oxidative stress improvement characterized by high levels of free thiol groups and reduced concentration of serum free radical. In addition, the glycemic profile remained stable in all subjects for the whole period, and the total antioxidant status was unmodified by ROC administration.

Despite the numerous preclinical studies demonstrated the useful of *Citrus* flavanones in diabetes, further human studies focused on dosage, bioavailability, efficacy, and safety are required.

8.5.3 Cancer and *Citrus* Flavanones

A growing amount of literature data evidences that the risk of cancer can be reduced with a diet rich in vegetables and fruits (Boffetta et al. 2010). Some epidemiological studies reported that *Citrus* fruit consumption is protective against a variety of human cancers. *Citrus* fruit exerts this chemopreventive action through the protection of DNA from injury and the inhibition of the early phase of carcinogenesis by promotion of xenobiotic detoxification process.

A recent review of Italian and Swiss case-control studies (10,000 cases of 14 different cancers and about 17,000 controls) has shown that the high consumption of *Citrus* fruit is associated with a reduced risk of cancers of the digestive tract and larynx (Turati et al. 2015). Previously the analysis data from a series of case-control studies regarding *Citrus* fruits intake and risk of several types of cancer has found that the ORs for the highest versus lowest category of *Citrus* fruit consumption varied from 0.42 to 0.82 for esophageal, oral cavity, pharyngeal, laryngeal, stomach, and colorectal cancer. Instead, the authors have found no consistent association with breast, endometrial, ovarian, prostate, and renal cell cancer. This observed protective effect against cancers of digestive and upper respiratory tract is linked to the content on flavanones, vitamin C, and other compounds with antioxidant, antimutagenic, and antiproliferative properties contented in *Citrus* fruit (Foschi et al. 2010). In addition, the meta-analysis done by Bae and Kim confirm the association between intake of *Citrus* fruit and gastric cancer risk evidencing a 13% reduction of gastric cancer. In particular, the authors reported that 100 g of *Citrus* fruit intake per day inhibits cardia gastric cancer by 40% (Bae and Kim 2016). *Citrus* flavanones such as naringin, hesperidin, and 20-hydroxyflavanone (2HF) also have antitumor activities. They inhibit tumor growth and promote cancer cell apoptosis through cell cycle arrest and caspase-activation induced by either death receptors or mitochondrial pathways, as demonstrated by both in vitro and in vivo studies. All these studies show the potential anticancer activity of *Citrus* flavanones even if presently no clinical studies support these findings and the current intake of *Citrus* fruits is insufficient to induce apoptosis of cancer cells in humans.

8.5.4 Neurodegenerative Disease and *Citrus* Flavanones

Neurodegenerative disorders including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and Huntington's disease are debilitating, incurable diseases that are becoming increasingly prevalent (Wyss-Coray 2016). Much attention is paid to dietary supplementation for maintaining cognitive function in old age and delaying the onset of neurodegenerative diseases. Epidemiologic studies reveals that higher intakes of flavonoids over 10–15 year are associated with a reduced rate of cognitive decline in old age (Kesse-Guyot et al. 2012; Letenneur et al. 2007).

Most of the human studies focused on flavanols and anthocyanins (Krikorian et al. 2012), while few researches have been performed with flavanones, even though they are the main components of the most commonly consumed juices and also are easily absorbed and cross the blood-brain barrier (Manach et al. 2005). A cross-sectional epidemiologic survey of 1091 men and women born in 1936 reported a positive association between flavanone consumption and crystallized intelligence (Butchart et al. 2011). Kean et al. (2015) carried out a controlled, placebo-matched, crossover, randomized, double-blind human-intervention trial on healthy older adults ($n = 37$) that drunk daily a flavanone-rich orange juice (305 mg) for 8 weeks. They reported an improvement of global cognitive function compared to low-flavanone control, while no difference in mood and blood pressure was observed. These findings underlined that constant daily flavonoid intake provides cognitive benefits not only to adults with mild cognitive impairment or neurodegenerative disease but also to healthy older adults. Up to date, no human studies provide data to support possible mechanisms that underlie positive cognitive effects of flavanones. Future research should be conducted to clarify the potential role of flavanone-based dietary supplementation to maintain and increase cognitive function in healthy young adults and mitigate cognitive impairment in older adults with neurodegenerative disease.

8.6 Application in Food

Many *Citrus* fruits, including particularly orange, clementine, tangerine, and grapefruit, are eaten fresh, unlike more acidic fruits such as limes and lemons that are generally not eaten fresh. In fact, generally, lemonade or limeade are beverages that are prepared by diluting derived juices and by adding sugar. *Citrus* juices and rinds are used in different drinks. The colorful peel of some *Citrus* fruits is used in cooking as a flavoring.

Although the consumption of *Citrus* fruits is reported since ancient times, *Citrus* processing was not possible until the development of both thermal and concentration processes. Since then, the *Citrus* industry has quickly developed, becoming soon prominent among food industries. Now, the *Citrus* industry is the second largest fruits processing industry, after the grape industry that principally produces wine. Approximately 30% of *Citrus* fruits is processed to obtain juice.

The variation of *Citrus* flavanones content in beverages and foods is dependent on different factors including *Citrus* species and cultivar, growth conditions, fruits' ripeness, postharvest processing, cooking process, and storage conditions.

Cooking processes, including boiling, baking, frying or microwave, are responsible of numerous changes in physical characteristics and chemical composition of food matrix (Zhang and Hamauzu 2004). Ismail et al. (2004) found that thermal treatment decreased the total phenolic content in food matrix. Previously, Gil-Izquierdo et al. (2002) analyzed some processes used for the production of orange juice at industrial scale, namely, mild and standard pasteurization, concentration, freezing, and squeezing. Moreover, commercial squeezing was compared with domestic squeezing in order to evaluate the influences of these processes on orange juice composition. Taking into consideration flavanones, mild and standard pasteurization processes did not influence the flavanones content. A slight decrease of flavanones content in the soluble fraction was proved. Only didymin decreased with a percentage of 52%.

The freezing process produced a considerable decrease of flavanones content in the soluble fraction. Comparing commercially and domestically squeezed orange juice, commercial squeezing gives a major content of flavanones than domestic squeezing. In agreement with this study, Moura and de Sylos (2009) analyzed samples of tangor murcott and orange juice (a mixture of Valencia and Pera varieties) and evidenced that pasteurization and concentration processes of juice did not affect significantly the amount of flavones hesperidin and narirutin.

The flavanone hesperetin is used as food enhancer and sweetener in a wide variety of dessert and alcoholic beverage (Ley et al. 2005).

Naringin is listed in Commission Decision 1999/217/EC establishing a register of flavoring substances in application of Regulation EC 2232/96, allowing its use in food without restriction. It has been subsequently evaluated by EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) in the Flavouring Group Evaluation 32 (EFSA 2011) with an interim finding that it is considered safe for use in food but that more data on human intake is desirable. A further possible application of naringenin in food matrix regards the inhibition of genotoxic compounds produced at 100–180 °C in particular acrylamide, which is dangerous for human health (Cheng et al. 2009). More than 30 years ago, Horowitz and Gentili (1971) reported that the peels of oranges and lemons contained a number of compounds, which could be converted into sweeteners.

8.7 Safety: Toxicity and Side Effects

Numerous studies demonstrated as *Citrus* flavanones are safe and well tolerated. Among these, particularly naringenin and hesperidin are investigated.

EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has assessed naringin in 2010 as a food flavoring. Data from chronic oral feeding studies in rats showed that naringenin up to 5 mg/kg complete feed is safe with an appreciable margin of safety.

Based on mammalian studies, only transient amounts of naringin and its metabolites residues in animal tissues are to be expected. This would represent an unimportant contribution to human exposure. Therefore, the use of the flavanone in animal's nutrition is evaluated safe for the consumer. However, the EFSA's panel on additives and products or substances used in animal feed (FEEDAP) could not conclude on the safety and efficacy of the product when delivered in water for drinking because related data are not available (EFSA 2011).

Li et al. (2014) investigated the potential chronic toxicity in Sprague-Dawley (SD) rats of naringin by oral gavage for 6 months followed by a recovery period of 1 month at doses of 0, 50, 250, and 1250 mg/kg. During both these periods, no mortality and/or toxicologically substantial variations in hematology, biochemical analyses, organ weights, ophthalmology, serum sex hormone, and histopathological and macroscopic examinations were reported. A slight, reversible, and non-pathological hair loss was observed during the treatment. However, it was not considered to be of toxicological importance. Overall, the obtained results demonstrated that the levels with no adverse effects of naringin, when orally administered in rats for 6 months, are greater than 1250 mg/kg/day.

Several animal and human studies have been reported that hesperidin is safe and well tolerated (Meyer 1994). The hesperidin safety was demonstrated on patients with rheumatoid arthritis administered 3 g of glucosyl hesperidin (G-Hsd) every morning for a 3-month treatment (Kometani et al. 2008). In another work, 94 menopausal women had a daily intake of 0.9 g of hesperidin with 0.3 g of hesperidin methyl chalcone and 1.2 g of vitamin C for 1 month (Smith 1964).

One of the most popular formulation containing hesperidin is Daflon 500 mg. This formulation has been analyzed in animals and humans trials (Meyer 1994). In animal studies, the safety of Daflon 500 mg is demonstrated by (i) a LD_{50} (lethal dose 50) of 3 g/kg, that is, 180 times the daily therapeutic dose, and (ii) by the absence of toxic effects after repeated oral dosing for 13 and 26 weeks, using a dose that represents 35 times the daily dosage in the rat and primate, respectively. The passage into breast milk and the transplacental passage are negligible. Clinical trials have collected more than 2850 patients treated with Daflon 500 mg at the dosage of 2 tablets/day for the period 6 weeks–1 year and satisfied the international scientific requirements. Side effects are essentially of a gastrointestinal or autonomic nature. Hemodynamic parameters as well as laboratory parameters were uninfluenced even by prolonged treatment for 1 year. Satisfactory clinical acceptability already confirmed in the short term was equally found in long-term treatment.

In animal studies, the concomitant administration of hesperidin with β-adrenergic blocking agents, calcium channel blockers, or statins significantly changed the maximal plasma concentration and the absorption of these drugs (Cho et al. 2009; Piao and Choi 2008; Uesawa and Mohri 2008).

In the study of Cho et al. (2009) the effects of hesperidin on the bioavailability and pharmacokinetic parameters of diltiazem and its desacetyldiltiazem (its main active metabolite) in rats, were investigated. Diltiazem (15 mg/kg) was orally administered in the presence or absence of hesperidin (1, 5 or 15 mg/kg), administered 30 min before the calcium channel blocker. Compared with the control group,

hesperidin (at doses of 5 or 15 mg/kg but not at 1 mg/kg) considerably modified the pharmacokinetics of diltiazem. Hesperidin (15 mg/kg) significantly increased the C (max) of desacetyldiltiazem but not significantly modified the desacetyldiltiazem metabolite-parent *ratio*. In conclusion, data from this work demonstrated the ability of hesperidin to enhance in rats the bioavailability of diltiazem probably by increasing its absorption and by reducing its first-pass metabolism in the intestine and in the liver via inhibition of cytochrome P450 3A or P-glycoprotein.

8.8 Marketed Products

Greater attention to health and social changes has led to the growth of the natural products market not only in the pharmaceutical and nutraceutical sectors but also in the food sector in Europe and in United States. This growth trend has been facilitated by the updated Novel Food legislation that makes easier for exporters to introduce new products. Sustainability in terms of reutilization of plant and food by-products is still a strong trend, demanded by companies as well as consumers (https://www.cbi.eu/market-information/natural-ingredients-health-products/trends/).

Citrus fruits represent a rich source of nutrition and vitamins and are important additions to any diet lacking in this components. *Citrus* are one of the key products in both food and beverage. Due its high nutritional value, *Citrus*-derived compounds could be used in innovative health functional food and beverage. Among *Citrus* flavanones, hesperetin, naringenin, eriodictyol, isosakuranetin, and their glycoside are the main abundant compounds. The *Citrus* flavanones are marketed individually or in the form of a mix, as nutraceuticals, since they act synergistically with the vitamin C to neutralize free radicals and counteract oxidative stress. The biological activities of *Citrus* flavanones are thought to be particularly beneficial for capillary strength, probably due an action on collagen (Kawaguchi et al. 2006). For this reason, these supplements are used in treatment of venous diseases such as chronic venous insufficiency, varicose veins, spider veins, and hemorrhoids (Mastantuono et al. 2015).

Moreover, hesperidin and naringenin are natural aromatase inhibitors. This activity is linked to a reduction in estrogen circulation that inhibited platelet aggregation and positively affect blood-clotting mechanisms which reduces the risk of cardiovascular disease. Bioflavonoids from *Citrus* possess also powerful anti-inflammatory properties (Benavente-García et al. 1997).

Recently, a researcher demonstrated the effect of naringenin on metabolic syndrome and obesity (Alam et al. 2014). Consequently, food supplements based on these bioactive compounds have been developed for this specific indication. Although the optimum daily doses of *Citrus* flavanones have not been determined, the label of nutraceutical products recommend 2000–6000 mg of these compounds for adults each day. This dose take into account the low toxicity of flavanones and the rare occurrence of side effects following their intake (Panche et al. 2016).

In food industry, hesperetin was used to fortified foods. A recent study proposed the nano-incapsulation of this flavanone in nanostructure lipid carriers coated with

different biopolymers such as alginate, chitosan, and low methoxypectin. Physico-chemical and sensorial analysis evidenced that developed nanoparticles could be applied for milk fortification to mask flavanone bitterness, inhibit color change, and enhance its solubility (Fathi and Varshosaz 2013). Further studies are necessary to identify other possible applications in food and nutraceutical industries.

8.9 Patents

The available information on patents was collected from some databases such as Espacenet (European Patent Office), Google Patents, and SciFinder. The research focuses on *Citrus* flavanones alone or as active ingredient in formulations used for the treatment of several pathological conditions. The search was performed in January 2019. The used keywords were: *Citrus* flavanones, naringenin, hesperetin, hesperidin, didymin, eriodictyol, neoeriocitrin, neohesperidin, poncirin, didymin, eriocitrin, and narirutin. A very high number of patents was found. For this reason, the research was limited to the last year (2018). After the removal of duplicated patents, the documents found were processed individually in an attempt to classify them based on the date and purpose for which they were registered (Table 2). Most of the patents found for the year 2018 do not concern single flavanones but are related to extracts or to mixtures of compounds (including flavanones) or to formulations in which are present flavanones.

Most of the patents have been designed and registered with the purpose of creating new products and formulations with specific biological activities. In particular, products with anti-inflammatory, and neuroprotective activity, products useful for the prevention and/or treatment of dermatitis, and skin damage caused by radiation and for repelling mosquitoes were prepared. Instead, fewer patents are present with regard to extraction and identification techniques. Among these, the patent CN108490094A reported the method for the determination of 22 flavonoids and phenolic acids in *Citrus* fruits.

8.10 Future Perspectives

Being *Citrus* flavanones daily present in the diet, their impact on human health is of relevance.

Extensive studies were carried out in order to investigate their ability to promote health and to provide protection against chronic diseases with high social impact including cardiovascular diseases, diabetes, and obesity. Among these compounds, particularly hesperetin, hesperidin, naringenin, naringin, and eriodictyol are mainly investigated.

In this chapter, we summarized and analyzed data related to the bioactivity of *Citrus* flavanones, their bioavailability and metabolism, their application in foods, marketed products, and safety.

Table 2 Patents overview of *Citrus* flavanones in 2018

Title	Date	Patent no.	Application no.	Use
Co-crystal of isoniazid and naringenin and preparation method of co-crystal	2018-09-28	CN 108586332A	CN 201810359523	Improvement of naringenin solubility and isoniazid activity
Application of naringenin and derivatives in the prevention and treatment of Alzheimer's disease	2018-11-13	CN 108785301A	CN 2018-11042921	Neuroprotective
Application of naringenin and naringin in tumor radiotherapy	2015-09-30	CN 104940932A	CN 2015-10404716	Tumor radiotherapy
Flavonoids pharmaceutical compositions and preparations and applications	2018-10-26	CN 108704037A	CN 2018-10626248	Prevention and treatment of dermatitis and skin damage caused by radiation
Mosquito repellent liquid	2018-04-21	CN108391665A	CN 201810363142	Repelling mosquitoes
Antibacterial coating and preparation method of green	2018-09-21	CN108559343A	CN 201810445336	Antimicrobial
Method for the determination of 22 *Citrus* fruit flavonoids and phenolic acids species	2018-09-04	CN108490094A	CN 201810274708	Compounds determination
Naringin microsphere silk fibroin/ hydroxyapatite composite scaffold and preparation method	2018-06-15	CN108159502A	CN 201810184370	Application to bone defect areas of different shapes
Application of naringin to preparation of medicine for treating and delaying intervertebral disc degeneration	2018-07-13	CN108272812A	CN 201810122205	Treating and delaying intervertebral disc degeneration
Such compositions and their use in the preparation of anti-inflammatory drugs	2018-09-04	CN108478549A	CN 201810497584	Anti-inflammatory

(continued)

Table 2 (continued)

Title	Date	Patent no.	Application no.	Use
Application of hesperetin, tangeretin and glycyrrhetinic acid in the inhibition of chloride channel	2018-11-16	CN108815154A	CN 201810866735	Treatment of diarrhea, heart, lung, gastric, brain, psychotic and ocular disorders, and rhinitis
Narirutin with effect of restraining allergic asthma	2018-05-25	CN108066353A	CN 201810138317	Effect of restraining allergic asthma
Whitening moisturizing skin care product	2018-06-22	CN108186496A	CN 201810199635	Whitening and moisturizing effects

The available data from animal models and clinical trials together with epidemiological studies suggest that this class of phytochemicals may beneficially affect both aetiology and physiopathology of several diseases. However, despite the ever-increasing number of studies on this flavonoids class, their health effects are still unknown or partially known. This is due to various reasons: (a) often interpretation of results from performed studies is problematic, because several different mechanisms and pathways may be involved; (b) serum biomarkers together with data from dietary intake could be used when studying the biological properties of *Citrus* flavanones; (c) clinical studies that investigate both high and low doses of *Citrus* flavanones are warranted; (d) bioavailability of each compound of a mix should be monitored, because marked interindividual variation could confound results; and (e) information about daily intake and bioavailability needs to be more fully developed.

8.11 Cross-References

- ▶ Dietary Flavonols and O-Glycosides
- ▶ Prenylated Flavonoids in Food
- ▶ Soy Isoflavones

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Chalcones in Diets

9

Siau Hui Mah

Contents

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Abstract

Chalcones are plant-derived polyphenolic compounds commonly found in edible plants such as tomatoes, apples, licorice, and fingerroot. The molecular structure of two aromatic rings connected by three carbons with conjugated carbonyl group and double bond results in the yellow or orange color of chalcones. The compound is biosynthetically available through chalcone synthase pathway and structurally related to flavanone and anthraquinone. A wide range of biological activities were found to be associated with natural chalcones from food and these activities havebeen proven to be contributed by the keto-ethylenic group in the main structure of chalcones. The biological activities reported include antioxidant, antimicrobial, anticancer, anti-inflammatory, antihypertensive, antidiabetic, anti-obesity, anti-neuroprotective, and hepatoprotective effects. Moreover, these chalcones have excellent profile in terms of food safety by showing non-toxicity against normal cells through a number of animal and human clinical studies. The overall beneficial effects of chalcones revealed that these compounds are highly nutritious and safe to be consumed, as well as highly potential lead compounds to be further developed into alternative drugs to treat various diseases.

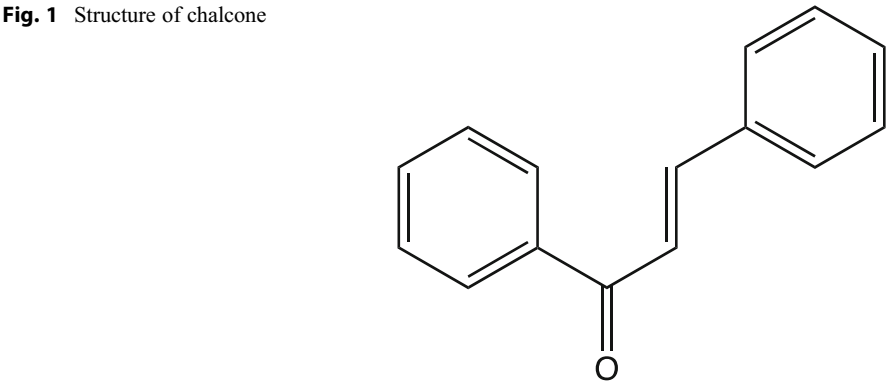
Keywords

3-Diphenyl-2-propen-1-one · Naringenin chalcone · Phloretin · Phloridzin · Isoliquiritigenin · Licochalcone · Cardamonin · Panduratin A

9.1 Introduction

Chalcone is a naturally derived compound present abundantly in edible plants, including vegetables, fruits, spices, and teas. It is widely distributed in various parts of the plant, including roots, rhizomes, leaves, and seeds. Examples of food sources that contain plenty of chalcones are tomato, apple, licorice, fingerroot, kava, wax jambu, and ashitaba leaves. These compounds are usually present as yellow or orange pigments in plants due to conjugated bonds in their structures. The word "chalcone" was first introduced by Kostanecki and Tambor in 1899 based on their discovery of mono-oxychalcone (Kostanecki and Tambor 1899). Chalcones are prominent secondary metabolites and serve as important precursors in the biosynthesis pathway of flavone-based compounds such as flavonoid and anthraquinone. As shown in Fig. 1, it has a unique core chemical structure, which is made up of two aromatic rings connected by a three-carbon linker consisting of α, β-unsaturated carbonyl group (–C=O–CH=CH–). As such, it possesses conjugated double bonds and delocalized π-electron system in the two rings and has a systematic IUPAC name of 3-diphenyl-2-propen-1-one. Chalcone has the tendency to exist in either *cis*- or *trans*-configuration due to the presence of double bond in the structure and is nearly planar in shape. The structure of chalcone as described gives rise to several alternative names such as benzylidene acetophenone, benzyl acetophenone, and *β*-phenylacrylophenone.

Fig. 1 Structure of chalcone



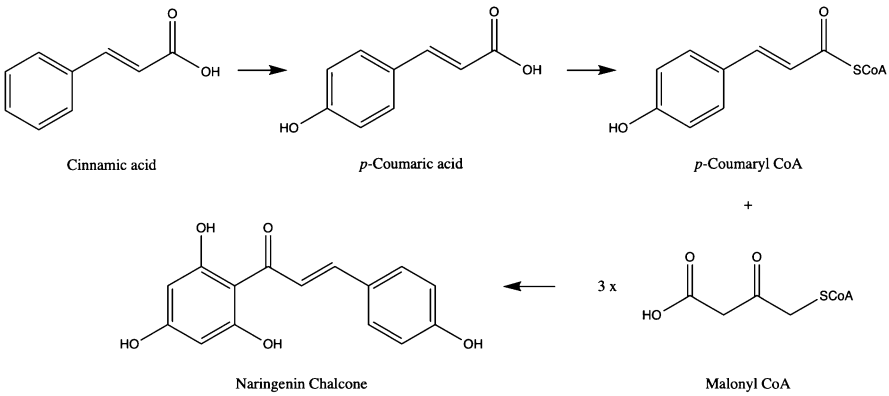


Fig. 2 Chalcone synthase reaction

The interesting scaffold of chalcones in terms of its core chemical structure has attracted numerous researchers worldwide to study on their derivatives and biological activities. Natural chalcones were produced through biosynthesis pathway of chalcone synthase, as presented in Fig. 2. Briefly, the biosynthesis reaction involves the condensation between a coenzyme A (CoA)-ester (starter substrate) of cinnamic acid or its derivatives such as coumaric acid or ferulic acid and three units of malonyl-CoA (extender substrate) by decarboxylation reaction. The product formed is a linear polyketide chain, which is further cyclized through intramolecular Claisen condensation and aromatization to give naringenin chalcone (Dao et al. 2011b; Jez et al. 2001). Naturally derived chalcones usually have hydroxylated aromatics rings. Common plants found to produce chalcones are *Acacia* (Hudson and Lewis 1983), *Angelica* (Kozawa et al. 1978; Baba et al. 1990; Nakata et al. 1999), *Carthamus* (Obara and Onodera 1979), *Myrica* (Mathiesen et al. 1996), *Coreopsis* (Geissman 1941; Kim et al. 2019), *Cryptocarya* (Fujioka et al. 2010), and *Desmos* (Nakagawa-

Goto et al. 2005). On the other hand, chalcones can be synthesized in chemistry laboratory through a general reaction of Claisen-Schmidt condensation, where aromatic aldehyde is condensed with either aliphatic or aromatic ketone in the presence of acid or base (Patil et al. 2009).

Both natural and synthetic chalcones have shown excellent biological properties with low toxicity profile. The presence of the keto-ethylenic group is believed to be the main contributor of these biological activities, with some studies confirmed that the removal of this group diminishes their activities. The keto-ethylenic group furnishes conjugation effect between the carbonyl group (–C=O) with its neighboring double bond (–C=C). Resonance occurs due to the side-by-side location of both functional groups and results in the free movement of electrons around these atoms. Consequently, resonance reduces the reactivity of the double bonds (–C=C) toward electrophilic additions but activates its ability for nucleophilic addition and thus the keto-ethylenic group is prone to nucleophilic attack. The mentioned reaction does not occur in the double bond (–C=C) that is located adjacent to other electron withdrawing groups such as amine (–CN), carboxylate ($–COO^-$), nitro ($–NO_2$), ester (–COOR), and ether (–COR) groups (Mayr et al. 2003; Yuvaraj et al. 2016). Hence, the keto-ethylenic group in chalcones is easily cyclized into biological active heterocyclic compounds through Claisen addition (1,2 addition) or Michael addition (1,4 addition), depending on the strength of the nucleophile and reaction conditions. For example, chalcones can be developed into pyrazoline, oxazoline, thiazine, oxazine, and pyrimidine (Hayat et al. 2010; Ingarsal et al. 2007; Selvam et al. 2013; Reddy and Reddy 2010; Kanagarajan et al. 2010).

A diverse biological activity has been found to associate with chalcone and its derivatives. The biological activities that have been reported are antioxidant (Wei et al. 2005; Dinkova-Kostova et al. 1998; Vogel et al. 2008), anticancer (Mendes et al. 2008; Modzelewska et al. 2006; Reddy et al. 2008; Sharma et al. 2010), anti-inflammatory (Zhang et al. 2014b), antihypertensive (Liu et al. 1992), neuroprotective (Han and Zhao 2010), antimicrobial (Nielsen et al. 2004; Boeck et al. 2005), anti-HIV (Wu et al. 2003), antitubercular (Lin et al. 2002), antimalarial (Li et al. 1995; Geyer et al. 2009; Mishra et al. 2008), anti-leishmanial (Kayser and Kiderlen 2001; Nielsen et al. 1995), anti-hyperglycemic (Enoki et al. 2007; Damazio et al. 2010), anti-tyrosinase (Khatib et al. 2005; Jun et al. 2007), anti-obesity (Horiba et al. 2010; Hassan et al. 2007), and vasorelaxant (Dong et al. 2010) activities. Furthermore, many studies have shown that both natural and synthetic chalcones show non-toxicity to normal cells (Utsintong et al. 2013; Sahu et al. 2012). The excellent pharmacological profile of chalcones revealed its potential to be developed as lead compounds for various diseases. In this chapter, the chalcones from food source will be the main focus. The most common chalcones found in edible plants are licochalcones (Furusawa et al. 2009), butein (Hudson and Lewis 1983; Geissman 1941), phloretin and its glucosidephloridzin (Lin et al. 2016; Mark and Larry 1993), and naringenin chalcones (Verhoeyen et al. 2002).

9.2 Bioactive Constituents (with Chemical Structures)

9.2.1 Tomato

Tomatoes (*Solanum lycopersicum*) are a common source of chalcones, especially naringenin chalcone (**1**) and its conjugates. It is a popular vegetable that is highly consumed worldwide and found to contain various polyphenols with health benefits. Flavanones, dihydroxy-cinnamates, and chalcones, together with their glycosides, are the main classes of polyphenolic compounds identified in tomatoes. Thus, tomato has been recognized as an important source of dietary flavonoids and chalcones, similar to prunes and citrus (Ross and Kasum 2002). A number of previous studies indicated that regular consumption of this vegetable is able to reduce the prevalence of cardiovascular diseases (Pandey et al. 1995; Silaste et al. 2007), diabetes (Lazarus et al. 2004), and cancer (Grieb et al. 2009; Zhang et al. 2009a), as well as improve perennial allergic rhinitis (Yoshimura et al. 2007; Iwamura et al. 2010). One of the predominant bioactive compounds present in tomatoes is naringenin chalcone (**1**), besides common flavonoids, such as rutin, naringenin, kaempferol, and quercetin. Other chalcones and its conjugates that were found in tomatoes are eriodictyol chalcone (**2**) (Iijima et al. 2008; Gómez-Romero et al. 2010) and dihydroxychalcones (Vallverdú-Queralt et al. 2011; Slimestad et al. 2008). These chalcones are produced almost exclusively in the skin or peel of the tomatoes with negligible quantity in other parts of the fruit (Slimestad et al. 2008). The accumulation of chalcones in tomatoes occurs with the onset of ripening, achieving maximum levels at the turning stage and followed by a rapid decrease during ripening. In other words, chalcones are not detectable in green peel, but its concentration increased steeply when turning into red tomatoes (Muir et al. 2001; Bovy et al. 2002). However, common processing methods of tomatoes in to ketchup usually transform this compound into naringenin, hypothesizing the degradation is correlated to temperature (Krause and Galensa 1992; Slimestad and Verheul 2005).

Naringenin chalcone (**1**) appears to be a major bioactive chalcone in tomatoes. It is regulated in a tissue-specific and development-dependent manner (Krause and Galensa 1992; Verhoeyen et al. 2002). This yellow pigment is formed simultaneously with the color turning of the fruit and accumulated in the cuticle of the tomato skin throughout fruit ripening (Verhoeyen et al. 2002; Ballester et al. 2010). The accumulation level of naringenin chalcone (**1**) at the colour turning stage is found to be approximately tenfold higher than that of rutin (quercetin 3-O-rutinoside), which is another major flavonoid present in tomato (Muir et al. 2001). Various studies have been conducted to investigate the bioactivities and health benefits of tomatoes in relation to this compound. The biological activities reported include antiallergic (Gafner et al. 2013; Yamamoto et al. 2004), anti-inflammatory (Hirai et al. 2007) and anti-obesity (Horiba et al. 2010; Hassan et al. 2007). The interesting biological profile of this chalcone has led to the exploitation of research studies on the chemical structure modifications to produce naringenin chalcone derivatives. One of the examples of derivative is tetracarboxymethyl-naringenin-

chalcone that showed inhibition toward the cathelicidin-induced inflammatory reaction in the chronic skin disease rosacea (Gafner et al. 2013).

For the antiallergic activity, naringenin chalcone (**1**) isolated from tomatoes showed inhibitory effects on histamine release. Interestingly, this chalcone showed the strongest inhibitory effects among the polyphenols present in the extract of tomato skin or peel. The inhibitory effects were not exhibited by flavonoid compounds, such as rutin, quercetin, and kaempferol (Yamamoto et al. 2004). In addition, this constituent is able to suppress airway inflammation in allergic asthma by inhibiting the Th2 cytokine production from CD4 T cells of the spleen. On the other hand, the anti-inflammatory activities of **1** have been proven in macrphage model by inhibiting the production of pro-inflammatory mediators such as tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, and nitric oxide (NO) in dose-dependent manner (Hirai et al. 2007). Naringenin chalcones (**1**) are also able to improve the adipocyte functions related to metabolic processes revealing its potential as an anti-obesity agent (Hassan et al. 2007; Kunimasa et al. 2009). The effect has been further confirmed in a study reporting on the exertion of insulin-sensitizing properties by activating an adiponectin-related pathway, enhancement on activity of peroxisome proliferator-activated receptor γ, and upregulation of the gene associated with mitochondrial energy metabolism by this constituent (Horiba et al. 2010).

A number of naringenin chalcone (**1**) and eriodictyol chalcone (**2**) conjugates were detected in tomatoes (Fig. 3). Similarly, these conjugates accumulated mainly in the peel of this health benefiting vegetable. Conversely, chalcone conjugates were found in the fruit of tomatoes. The common conjugate moieties seen to be associated with these chalcones are hexoses (*hex*). The conjugate moieties identified in eriodictyol chalcone (**2**) were also found to be present in naringenin chalcone (**1**). Interestingly, naringenin chalcone (**1**) has been reported to have a wider variety of conjugate moieties, including deoxyhexose, pentose, and malonylated hexose, and some unidentified moieties (Iijima et al. 2008; Mintz-Oron et al. 2008; Moco et al. 2006). Similar modification patterns in conjugation of eriodictyol chalcone (**2**) and naringenin chalcone (**1**) imply that these constituents share the same set of enzymes in their modification pathways. The biological activities of chalcone aglycone and its conjugates are very likely to be different from each other based on previous studies on the constituents with conjugates (Iijima et al. 2008; Nguyen et al. 2004; Yao et al. 2006).

The dihydroxychalcones found in tomatoes are phloretin-3′,5′-di-*C*-glucoside (**3**) and dihydroxy-dimethoxychalcone-*C*-diglucoside (**4**) with the structures presented in Fig. 3. Phloretin-3′,5′-di-*C*-glucoside (**3**) is the first *C*-glycoside, as well as first dihydrochalcone, identified in tomatoes. The presence of constituent **4** was reported only in tomato juices and ketchup but not in fresh tomatoes. The production of **4** is possibly due to the technological preparation processes of tomatoes such as dehydration and stabilization (Gahler et al. 2003; Galicia-Cabrera 2007; Vallverdú-Queralt et al. 2011). Once again, studies showed that the industrial processes of tomatoes into food products did play an important role in terms of preserving the nutritional constituents. Dihydrochalcones have been proven to show significant

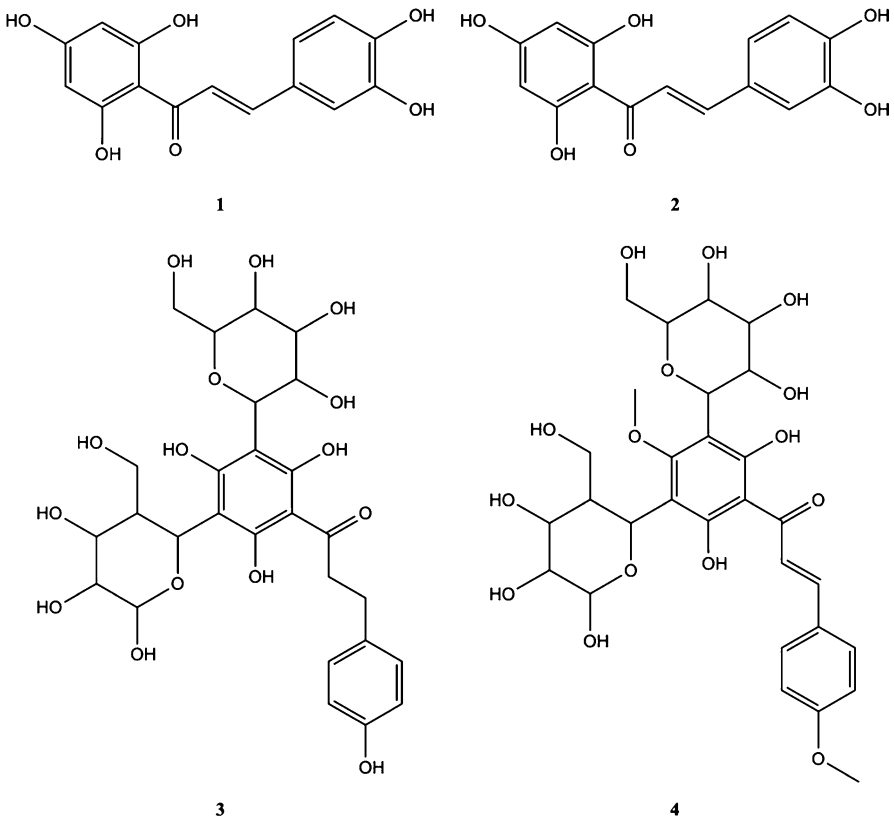


Fig. 3 Structure of naringenin chalcone (**1**), eriodictyol chalcone (**2**), phloretin-3′,5′-di-*C*-glucoside (**3**), and dihydroxy-dimethoxychalcone-*C*-diglycoside (**4**)

antioxidant activities in radical scavenging and lipid peroxidation suppression activities (Dziedzic et al. 1985; Nakamura et al. 2003). These constituents are widely applied in the food industry as food additives due to their stability, colorless property, safe consumption, and easy producibility by catalytic hydrogenation from flavanones and glucosides (Borrego et al. 1995).

9.2.2 Apple

Apple is a rich source of polyphenols such as flavonoids, hydroxycinnamates, anthocyanins, and dihydrochalcones (Ramirez-Ambrosi et al. 2013). Numerous studies have reported the polyphenols produced by apple are good antioxidants and have preventive effects against cardiovascular diseases and cancers (Sun and

Liu 2008; Yoon and Liu 2007; Reagan-Shaw et al. 2010; Sesso et al. 2003). Among the polyphenols, dihydrochalcones are the dominant constituents that accumulate in the root barks, shoots, leaves, and fruits of apples. For the fruits, dihydrochalcones build up in the tissues of apples, including skin, pulp, and specifically core and seeds that are usually discarded when the apples are consumed freshly. Thus, the overall intake of these chalcones has been claimed to be greater for apple-derived products such as juice, cider, and pomace since whole apple fruit is processed. Furthermore, fruit bruising will result in browning and 20–40% reduction on the amount of dihydrochalcones (Tomás-Barberán and Clifford 2000). Dihydroxychalcone is considered somewhat distinctive in plants since this chalcone is only traceable in approximately thirty plant families.

The main dihydrochalcone present is phloretin (**5**) and its conjugate phloridzin or phlorizin (**6**), which comprises up to 90% of the soluble polyphenols found in the leaves of the apple tree (Gosch et al. 2010b). Phloridzin (**6**) is also known as phloretin-2′-β-D-glucopyranoside, a glucoside of phloretin (**5**), as presented in Fig. 4. The amount of phloridzin (**6**) in apples varies greatly depending on the cultivar (Burda et al. 1998; Amiot et al. 1992; McRae et al. 1990). This white to yellow crystalline solid is sweet in taste and found in strawberries as well. However, both constituents **5** and **6** do not accumulate in the plant species closely related to apple such as pear and cherry, even though these species show strong similarities of flavonoid genes (Fischer et al. 2007; Williams 1964; Andreotti et al. 2006). Thus, these differences have been utilized for juice authenticity control (Schieber et al. 2001) and chemotaxonomic differentiation (Challice 1981). These constituents have wide applications in many industries such as natural sweetener, longevity extending products and food additives in food and beverages, cosmetics, and pharmaceuticals (Ehrenkranz 2006; Aratsu et al. 2003).

The biosynthesis pathway of phloridzin (**6**) has been claimed to be involved in the reduction reaction of *p*-coumaroyl CoA to dihydro-*p*-coumaroyl CoA and subsequently converted into phloretin (**5**) by the action of chalcone synthase pathway. The reaction is followed by the glycosylation of phloretin (**5**) (Jugde et al. 2008; Gosch et al. 2009, 2010a). The other dihydrochalcones that have been found in apples and its derived products are phloretin 2′-*O*-xylosylglucoside (**7**) and 3-hydroxyphloridzin (**8**) (Lu and Foo 1997; Burda et al. 1998) (Fig. 4). The human health beneficial effects of dihydrochalcones, especially phloretin (**5**) and phloridzin (**6**), have been exploited in the last decade with a number of patents being filed for treatment of neurological disorders and diabetes (Diamandis et al. 2008; Ehrenkranz 2006; Aratsu et al. 2003). These constituents have shown various biological activities such as antioxidant (Bors et al. 1990; Calliste et al. 2001; Rezk et al. 2002), antimicrobial (Vikram et al. 2010), anti-inflammatory (Lee et al. 2011), anticancers (Yang et al. 2001; Lin et al. 2016), and inhibition of cardiovascular disease (Stangl et al. 2005).

Phloretin (**5**) showed potent antioxidant activities in many bioassays, including lipid peroxidation inhibition, peroxynitrite scavenging, 1,1-diphenyl-2-picrylhydrazyl radical scavenging, and hydroxyl radical scavenging (Bors et al. 1990; Calliste et al. 2001; Rezk et al. 2002). The antioxidant activities of phloridzin (**6**) are much weaker

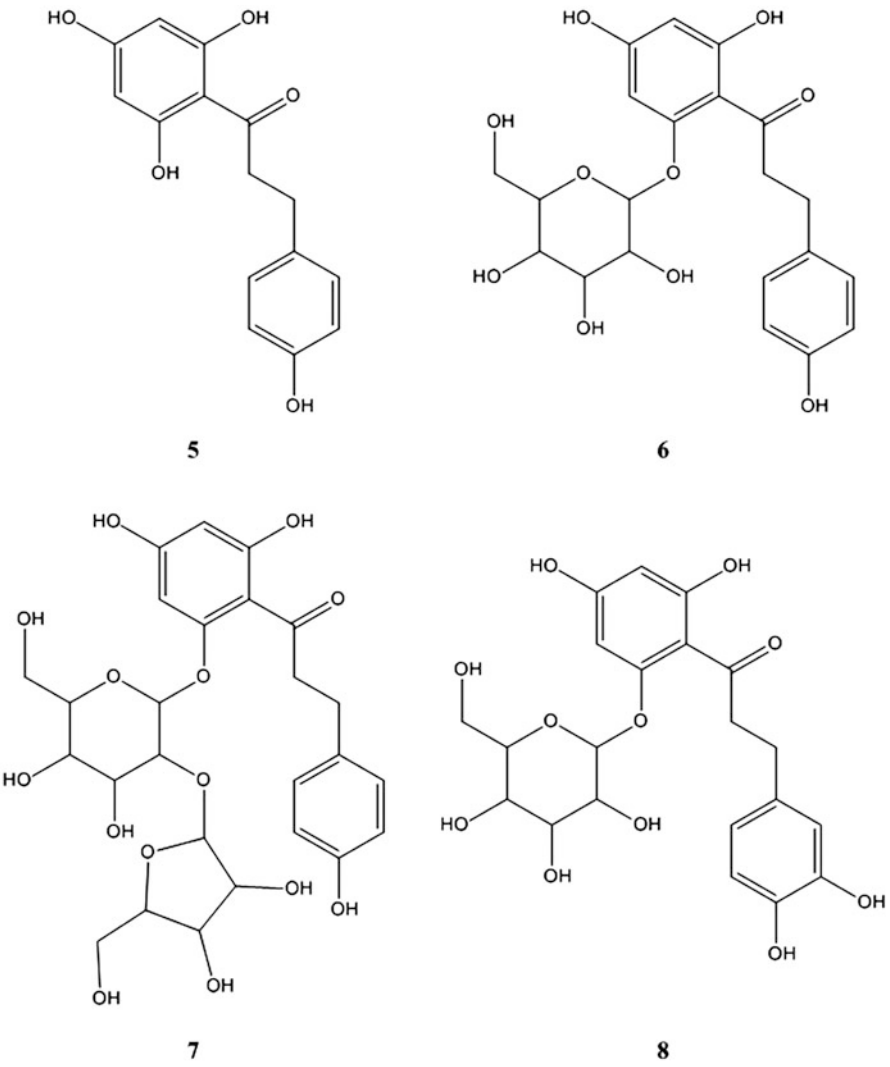


Fig. 4 Structures of phloretin (**5**), phloridzin (**6**), phloretin 2′-*O*-xylosylglucoside (**7**), and 3-hydroxyphloridzin (**8**)

if compared to **5**, indicating that hydroxyl group (-OH) of **5** being replaced by the glucose unit as seen in **6** is important in the contribution of activities (Rezk et al. 2002). Many studies have been done on the antitumor effects of phloretin (**5**), and most of them showed that this constituent has significant inhibitory effects on colon, bladder, liver, lung, and breast cancer cells (Delphi and Sepehri 2016; Schiavano et al. 2015; Wu et al. 2009; Yang et al. 2009; Nelson and Falk 1993; Ma et al. 2016). These studies demonstrated that **5** is a specific inhibitor of GLUT2, which will be expressed in a higher level in tumor tissues than normal cells, revealing its potential

to be used in cancer therapy. Besides, it also showed antitumor growth effects in breast cancer cells and metastasis mechanisms by inhibiting cell growth and arresting the cell cycle in breast cancer cells in a p53 mutant-dependent manner, as well as decreasing the migratory activities of the cells (Wu et al. 2018).

Phloretin (**5**) has shown to give positive effects toward inflammatory bowel diseases (IBD). In addition, it is able to downregulate the mRNA levels of many pro-inflammatory cytokines such as NF-κB, TNF-α, interleukin-8, CXCL3, and CXCL10 in human colon epithelial cells (Jung et al. 2009). The outcome is further proven by another study on the suppression of TNF-α-induced inflammatory response in human colonic epithelial cells and amelioration of colon inflammation in animal model (Lee et al. 2011). The study also showed that constituent **5** is a nontoxic inhibitor of *E. coli* O157:H7 biofilms formation, revealing its potential to be used as a lead compound in treating IBD. Phloridzin (**6**) has also been proven to be a potential food additiive or dietary auxiliary agent for patients with ulcerative colitis, which is one type of the IBD, by improving the symptoms of acute colitis and protecting the intestinal brush border (Lu et al. 2018). It is also found to possess antipyretic (Gosch et al. 2010a), antidiabetic (Ehrenkranz 2006), and melanogenic stimulatory (Jung et al. 2009) properties.

9.2.3 Licorice

Licorice or liquorice plant is one type of legumes, which is rich in bioactive chalcones. It belongs to the family of Leguminosae where the representative species are *Glycyrrhiza* sp., such as *G. glabra*, *G. inflata*, and *G. uralensis* (Yang et al. 2017). Licorice is commonly used as a food ingredient due to its sweet flavors. Thus, this plant is widely used in the preparation of pharmaceutical products such as hematinic pills and expectorant in cough to mask the bitter taste of other remedies (Fenwick et al. 1990). The examples of licorice-containing products in the food industry are snacks, candies, beverages, as well as dietary supplements. It is also used by tobacco companies as a flavoring and moistening agent (Omar et al. 2012). Besides, the roots of licorice are commonly used as prescribed herbs in Chinese traditional medicines for a variety of ailments such as gastric ulcers, bronchitis, fevers, and sore throats (Isbrucker and Burdock 2006; Furusawa et al. 2009). It has been widely used as traditional medicine in the countries of Asia and Europe, including China, Japan, and the United Kingdom. It is commonly called as Gan Cao in China. Commercially, licorice exists in two principal forms, which are licorice root and licorice extract (Isbrucker and Burdock 2006).

Extensive studies on this plant revealed the existence of a high abundance of flavonoids. The principal metabolites of licorice roots are triterpene saponins, as well as flavanones and chalcones with a variety of conjugates (Isbrucker and Burdock 2006; Simmler et al. 2013). Glycyrrhizin is a triterpene saponin that is present in a significant amount in roots of licorice. It is 30–50 times sweeter than sucrose and has been widely used as an acceptable alternative sweetener by food producers and consumers (Omar et al. 2012; Yang et al. 2017). Furthermore, this compound has

been reported to be responsible for various pharmacological effects such as anti-inflammatory, antiviral, anticancer, and hepatoprotective properties (Ashfaq et al. 2011). On the other hand, the glucosides of flavanone (liquiritigenin) and 2′-hydroxychalcone (isoliquiritigenin) (**9**) and its isomer are also present as the main constituents in licorice (Nomura et al. 2002; Zhang and Ye 2009). These isomers are chemically interchangeable, and the interconversion occurs as a function of time and temperature, subsequently affecting the glycosylation of both constituents (Simmler et al. 2013). Interestingly, the glycosides of these compounds are claimed to be important in contributing the biological activities as either prodrugs or bio-equivalent agents of the corresponding aglycones, which are flavanone and isoliquiritigenin (**9**) (Kamei et al. 2005; Asl and Hosseinzadeh 2008).

Isoliquiritigenin (**9**), as shown in Fig. 5 has beneficial effects in the inhibition of NLRP3 inflammasome activation, which is an important factor in inflammatory diseases such as type 2 diabetes (Simmler et al. 2013; Honda et al. 2014). The treatment with this chalcone caused a reduction in high-fat-diet-induced obesity, hypercholesteremia, insulin resistance, blood glucose lowering, and anti-hyperglycemia effects (Honda et al. 2014; Gaur et al. 2014). Anticancer studies on compound **9** reported its promising capability in chemoprevention such as the ability to inhibit ErbB3 signaling and PI3K/Akt pathway in DU145 prostate cancer cells and regulate cell proliferation (Jung et al. 2006). Furthermore, the anti-invasive effect of isoliquiritigenin (**9**) was elucidated on MCF-7 breast cancer cells through a reduction in the secretion of vascular endothelial growth factors (VEGF), as well as inhibition of PI3K and NF-κB signaling pathways (Wang et al. 2013). The modulation of breast cancer cell proliferation through apoptotic induction by this chalcone was proven, and it increased PTEN expression to inhibit aberrant Akt signaling through modulation of the miR-374a/PTEN/Akt axis (Peng et al. 2017). Additionally, isoliquiritigenin (**9**) displayed potent antioxidant effects by reducing cardiac reactive oxygen species (ROS) levels in ischemic injury through the activation of AMPK and ERK signaling pathways (Chin et al. 2007; Zhang et al. 2013).

Licochalcones are the major chalcones present in the licorice plant, and the examples are licochalcone A (**10**), licochalcone B (**11**), licochalcone C (**12**), licochalcone D (**13**), and licochalcone E (**14**) with their structures presented in Fig. 5. These chalcones, especially licochalcone A (**10**), were found to possess various biological activities, including anti-inflammatory (Furusawa et al. 2009; Yang et al. 2017), anti-leishmanial, antibacterial, (Zhai et al. 1995; Tsukiyama et al. 2002), anticancer (Nabekura et al. 2015), antidiabetic (Park et al. 2012; Lee et al. 2018), and antiviral (Dao et al. 2011a) activities. Licochalcone A (**10**) exhibited inflammatory suppression activities by inhibiting a range of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. It has been proven capable of suppressing the iNOS gene expression and NF-κB signaling pathway, which leads to inflammatory response (Kwon et al. 2008; Furusawa et al. 2009). Moreover, this chalcone is able to inhibit Th2 cytokines IL-4, IL-5, and IL-23 and hypersecretion of mucus by goblet cells in the airway and subsequently resolve inflammation (Chu et al. 2013).

For antitumor activities, licochalcone A (**10**) induced apoptosis in PC-3 prostate cancer cells and suppressed cell cycle regulators such as cyclin B1 and cdc2 in G2

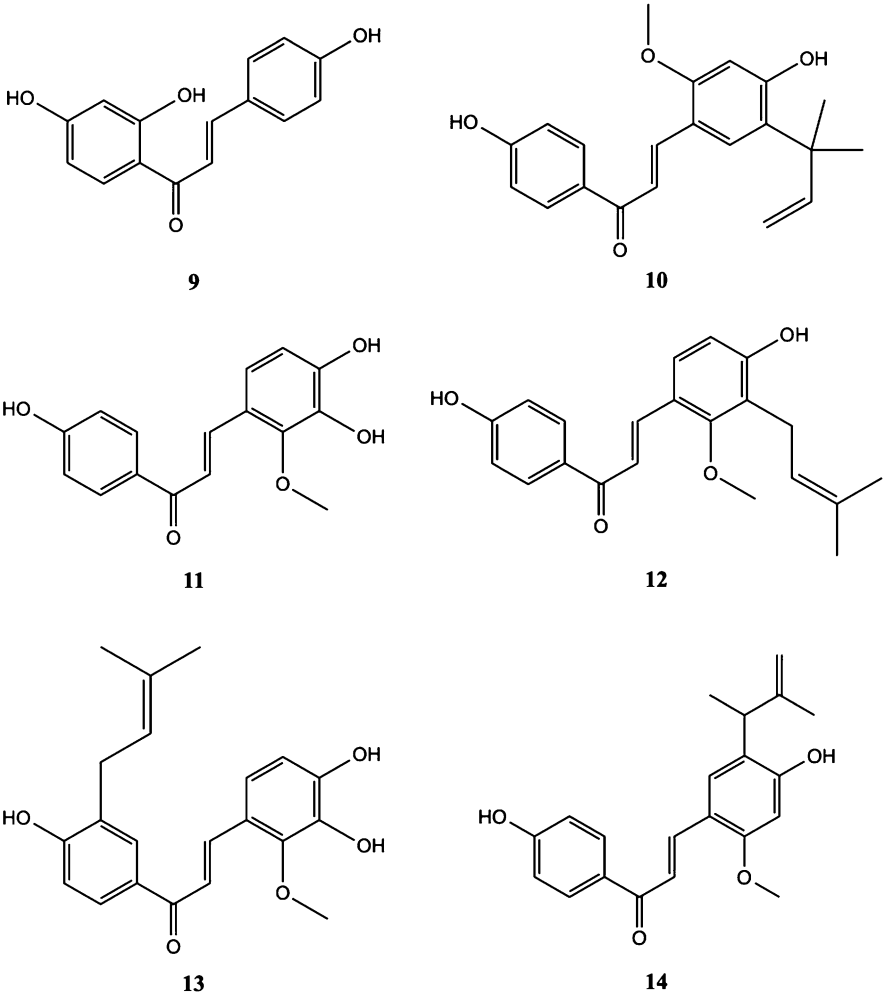


Fig. 5 Structures of isoliquiritigenin (**9**), licochalcone A (**10**), licochalcone B (**11**), licochalcone C (**12**), licochalcone D (**13**), and licochalcone E (**14**)

and late G1 phase of PC-3 prostate cancer cells (Fu et al. 2014). This chalcone also showed inhibitory activities in cell proliferation and apoptosis in DU145 cells (Park et al. 2014). Furthermore, the specific apoptosis induction in glioma stem cells by mitochondrial dysfunction but not in normal somatic and neural stem cells of this chalcone reveals its promising potential as an anticancer agent (Kuramoto et al. 2017). Further study on animal model showed that oral administration of licochalcone A (**10**) gave antitumor effect in a colitis-associated colon cancer model (Kim et al. 2010). On the other hand, compound **10** has been reported to possess antifungal activity against *Candida albicans* and significantly inhibited the

formation of biofilm, highlighting its potential as an antifungal agent for oral candidiasis (Messier and Grenier 2011; Seleem et al. 2016).

Licochalcone A (**10**) was also found to have the ability to enhance the anti-plasmodial activity of arteminisin, which is a well-known antimalarial drug. This study was done to counter the problem of antimalarial drug resistance by applying lichochalcone A (**10**) in the artemisinin-based combination therapies (ACTs), as well as the shortage of artemisinin. Thus, the interactions of arteminsinin and compound **10** were evaluated against synchronized erythrocytic stages of chloroquine-sensitive 3D7 and chloroquine-resistant RKL 303 strains of *P. falciparum*. Interestingly, this combination exhibited synergistic anti-plasmodial activity on these strains. Artemisinin was found to interfere with the hemozoin formation by the parasite but not the chalcone. Both compounds also did not show reduction in sorbitol-induced hemolysis, suggesting that these compounds did not inhibit the new permeation pathways (Mishra et al. 2009).

Licochalcones A–D (**10**–**13**), which are also known as retrochalcones, showed excellent antibacterial profile, especially lichochalcone A (**10**) and licochalcone C (**12**). Compounds **10** and **12** carry a prenyl side chain in their structure that makes the structures highly hydrophobic and able to penetrate the cell membrane, thus resulting in a strong effect in the inhibition of bacterial respiration in whole cells and NADH oxidase in the bacterial membranes (Haraguchi et al. 1998). On the other hand, licochalcone E (**14**) has been getting more attention with the studies focused on its anti-inflammatory properties through downregulation of NF-κB, revealing its potential in the treatment of chronic contact dermatitis (Cho et al. 2010). In addition, this chalcone exhibited antidiabetic properties through an increase in PPARγ expression that results in an increase in adipocyte differentiation and acts as a resolution of hyperglycemia and hyperlipidemia in diabetic patients (Park et al. 2012). Interestingly, licochalcone E (**14**) exhibited antimicrobial activity against *Staphylococcus aureus* strains and reduced the production of α-toxins, even in the methicillin-resistant strain, MRSA (Zhou et al. 2012).

9.2.4 Fingerroot

The tropical plant fingerroot (*Boesenbergia rotunda*) is also known as Chinese keys or Chinese ginger. Fingerroot belongs to the ginger family, Zingiberaceae, and is used as a flavoring agent or eaten as a vegetable. It is commonly used as a spice in Asian cuisine, particularly Thai dishes, and known as Krachai in Thailand (Trakoontivakorn et al. 2001). Rhizomes and leaves of species from this family are commonly associated with antioxidant properties and have also been used in traditional medicine (Chan et al. 2008). The popularity and wide applications of fingerroot have gained the interests of researchers and resulted in extensive studies reporting on various pharmacological activities such as antiallergic, antibacterial, anticancer, and anti-inflammatory properties (Kiat et al. 2006; Ongwisespaiboon and Jiraungkoorskul 2017). These activities were claimed to be contributed by chalcones and flavonoids present in the plant.

The bioactive chalcones found in fingerroot are boesenbergin A (**15**), pinocembrine chalcone (**16**), cardamonin (**17**), pinostrobin chalcone (**18**), panduratin A (**19**), and 4-hydroxypanduratin A (**20**) (Fig. 6). Each of these chalcones contributes to different pharmacological effects and concentrates in different ratios in

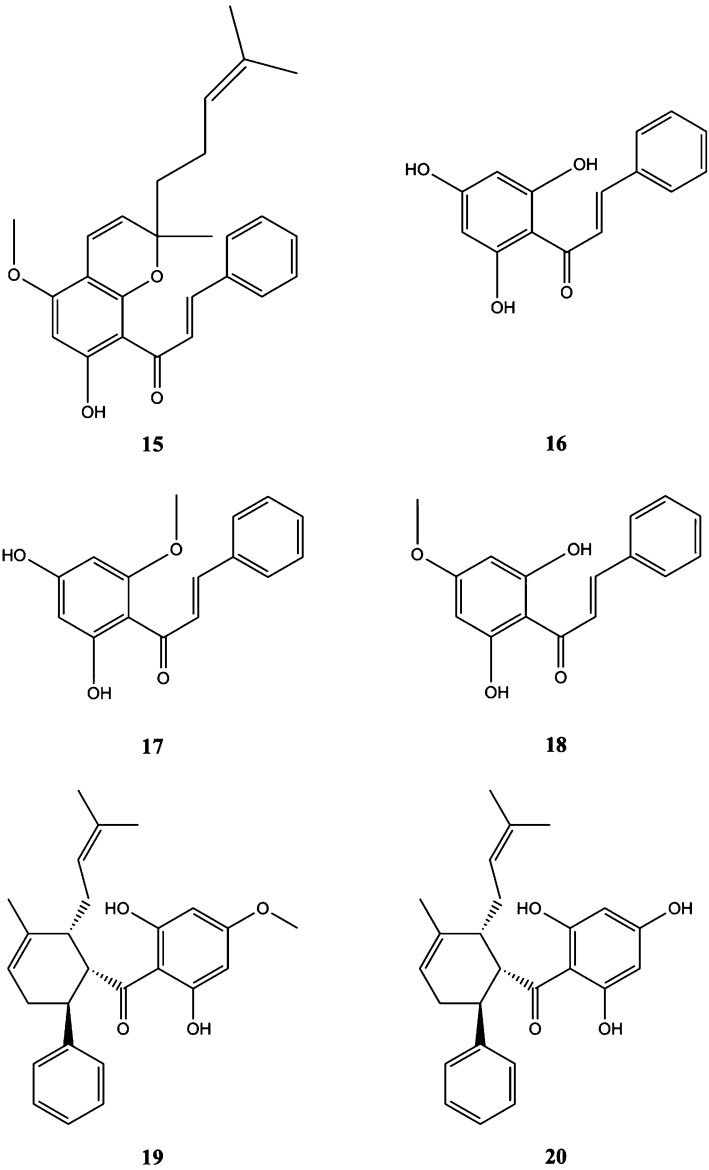


Fig. 6 Structures of boesenbergin A (**15**), pinocembrine chalcone (**16**), cardamonin (**17**), pinostrobin chalcone (**18**), panduratin A (**19**), and 4-hydroxypanduratin A (**20**)

various parts of the plant (Trakoontivakorn et al. 2001; Isa et al. 2012; Tan et al. 2015; Woo et al. 2015; Ongwisespaiboon and Jiraungkoorskul 2017). Fingerroot plant has been used traditionally for its aphrodisiac properties, and the effects were found to be associated with boesenbergin A chalcones (**15**), pinostrobin chalcone (**18**), and panduratin A (**19**) (Ongwisespaiboon and Jiraungkoorskul 2017). Besides, the antibacterial effect of the fingerroot was attributed to chalcones, pinocembrin chalcone (**16**), pinostrobin chalcone (**18**), and panduratin A (**19**). Compound **16** was found to act against *Staphylococcus aureus*, *Helicobacter pylori*, and *Salmonella typhimurium* (Bremner and Meyer 1998; Trakoontivakorn et al. 2001).

Panduratin A (**19**) and its derivative 4-hydroxypanduratin A (**20**) that contains a cyclohexene moiety are found in relatively high concentrations in fingerroot (Trakoontivakorn et al. 2001). Several biological activities have been reported on these chalcones, including anti-inflammatory, antioxidant, antibacterial, and anti-obesity properties (Kim et al. 2013; Woo et al. 2015). Compounds **19** and **20** demonstrated anticancer effects on breast (MCF-7), colon (HT-29), lung (A549), and prostate (PC3 and DU145) cancer cells through inhibition of cell proliferation and induction of apoptosis. Moreover, these chalcones are also associated with anti-inflammatory effects through inhibition of COX-2 enzymes (Kirana et al. 2007; Isa et al. 2012). The improvement of skin barrier function and modulation of Th1, Th2, and Treg-related immune responses were observed after treatment with panduratin A (**19**). The potential of panduratin A (**19**) as a skin nutraceutical, owing to its ability to enhance skin hydration and barrier function, was further confirmed by the modulation of cornified envelope formation and filament aggregating protein processing (Woo et al. 2015). Besides, this chalcone is a highly potential natural anti-biofilm agent that can be used to eliminate oral bacterial colonization during early dental plaque formation, attributed to its potent anti-biofilm properties against *Streptococcus mutans*, *Streptococcus sanguis*, and *Actinomyces viscosus* (Yanti et al. 2009).

Boesenbergin A (**15**) was found to possess anti-inflammatory and antioxidant properties (Isa et al. 2012). Similar to panduratin A (**19**), this compound exhibited cytotoxicity against several cancer cell types such as MCF-7, HT-29, and A549 cancer cells (Isa et al. 2012). However, in contrast to panduratin A (**19**), boesenbergin A (**15**) showed high toxicity against normal liver cells, WRL-68. It is hypothesized to be linked to higher metabolism of **15** in the cells, suggesting its potential side effects despite its anticancer activity (Isa et al. 2012). Another chalcone cardamonin (**17**) has gained a lot of attention in recent years due to its anticancer, antipyretic, and protease inhibitory activities (Tan et al. 2015). The selective inhibition of drug-resistant breast cancer stem cells and chemoprotective activity of **15** were reported. Besides, its anti-inflammatory effects were demonstrated on monocytes and macrophages through inhibition of nitric oxide (NO) release, as well as COX-2 and iNOS expression (Hatziieremia et al. 2006; Israf et al. 2007). This compound has also been reported to demonstrate inhibitory effect against HIV-1 protease (Cheenpracha et al. 2006; Kiat et al. 2006).

The HIV-1 protease inhibition activities were also exhibited by other chalcones present in fingerroot, pinostrobin chalcone (**18**), panduratin A (**19**), and hydroxypanduratin A (**20**) (Tewtrakul et al. 2003; Cheenpracha et al. 2006). Studies

revealed that cardamonin (**17**) and pinostrobin exhibited noncompetitive inhibition toward dengue 2 virus NS3 protease, while competitive inhibition was shown by panduratin A (**19**) and 4-hydroxypanduratin A (**20**) (Kiat et al. 2006). In addition, compound **17** showed apoptotic induction and inhibition of fibrosarcoma growth, further highlighting the medicinal role of fingerroot for its anticancer benefits (Nurrachma et al. 2018). Cardamonin (**17**) also exhibited hepatoprotective effects against ischemia reperfusion injury in rats model by elevation of NO/eNOS and reduction of iNOS. Treatment with cardamonin (**17**) could be a promising strategy to improve functional and structural abnormalities of the liver in patients (Atef et al. 2017).

9.3 Bioavailability and Metabolism

One of the factors that are involved in the bioavailability and metabolism of food phenolic compounds is the absorption of compounds that have sugar or organic acid and ester linked substitutions in their structures. This specific feature allows the compounds to be degraded by the esterase enzymes produced in the colon and unabling them to be reabsorbed into the upper part of the gastrointestinal tract (Scalbert and Williamson 2000; Olthof et al. 2001; Rechner et al. 2001; Adam et al. 2002; Rondini et al. 2002). In addition, the number and type of sugar unit present in the phenolic compounds also play important roles in their absorption. Sugar units such as glucose, galactose, or xylose that are attached to the phenolic compounds are able to be absorbed through the small intestine by the cytosolic β-glucosidase or lactase phlorizin hydrolase but not rhamnose due to its degradation by the enzymes of rhamnosidase. For example, dihydroxychalcones are absorbed in the small intestine of rats followed by conjugation reaction; thus their glycosides could be recovered intact in plasma (Crespy et al. 2001a, b; Glöck et al. 2001).

The metabolism of dietary phenolic compounds is done by deconjugation and reconjugation processes, whether the compounds are hydrolyzed into aglycones and conjugated by methylation, sulphation, and/or glucuronidation. Similar to pharmaceutical agents, food phenolic compounds can act as substrates for both phase I and II metabolic enzymes. The most common example of phase I enzyme is cytochrome P450, while phase II enzyme is sulfotransferases or 5′-diphosphoglucuronyl transferases (UGTs). Phase I metabolism is very likely to produce metabolites with enhanced pharmacological activities or toxicity. Conversely, phase II conjugation reactions might result in metabolites with reduced activities and have higher chances to be excreted. As mentioned previously, chalcones, which are obtained from food sources, have been widely studied for their pharmacological activities. However, the studies on the bioavailability and metabolism of these metabolites are limited.

The oral bioavailability of the well-known chalcone naringenin chalcone (**1**) from tomatoes was studied using the rat model, and this chalcone was found to be metabolized extensively by glucuronidases. Three major metabolites, naringenin chalcone-2′-O-β-D-glucuronide, naringenin-7-O-β-D-glucuronide, and naringenin-4′-O-β-D-glucuronide, were detected in urine, while only one of these metabolites

which is naringenin chalcone-2′-O-β-D-glucuronide was found in plasma after 1 h of administration of **1**. This metabolite was then found to inhibit histamine production, suggesting its contribution to antiallergic activities of naringenin chalcones (**1**) (Yoshimura et al. 2009). However, some studies indicated that the metabolites might be deconjugated during inflammation and contribute to its aglycone bioactivity (Kawai et al. 2008). Thus, the antiallergic activity might be contributed by naringenin chalcone (**1**) instead of its metabolite; study on the active form of metabolites in the inflammatory site is highly recommended.

Phloretin (**5**), a dihydroxychalcone present exclusively in apples, and its glycoside product phloridzin (**6**) were studied for their bioavailability and metabolism because these constituents were commonly consumed. Phloridzin (**6**) was found to be hydrolyzed to its aglycone, phloretin (**5**) and glucose by lactase-phloridzin hydrolase (LPH) in the brush border of small intestine in the hamster (Malathi and Crane 1969). A detailed study of the bioavailability of phloretin (**5**) further showed that approximately half of the doses were excreted in urine within 2 days after phloridzin (**6**) was administered in the rats. Trace amount of **5** was still detected, but most of the metabolites were degraded products. Examples of metabolites are phloroglucinol and phloretic acid, together with their related metabolites produced through dehydrogenation, β-oxidation, and glycine conjugation processes. The results were further confirmed that phloretin (**5**) was hydrolyzed to phloretic acid and phloroglucinol by the cecal microflora in rats, which were discovered in urine and feces (Monge et al. 1984). Interestingly, phloretin (**5**) was also detected in urine, suggesting that it was absorbed before metabolism.

A confirmation study with high-dose feed of phloretin (**5**) and phloridzin (**6**) in rats was conducted. These chalcones showed different absorption rate with phloretin (**5**) appeared to be absorbed more rapidly in plasma than phloridzin (**6**). However, both chalcones were absorbed rapidly in the intestine with no trace in plasma at 24 h, indicating an efficient excretion in urine (Crespy et al. 2001b). Moreover, another study was done by administering phloridzin (**6**) into rats by intravenous (IV) cannula at a concentration of 5μM. The results demonstrated that the uptake of glucose by active glucose transporter SGLT1 in the small intestine was inhibited and subsequently impaired the resorption of glucose in kidney and resulted in glucosuria. This study indicated that phloridzin (**6**) is diabetogenic, but this is a misinterpretation due to lack of the evidence on the effects in the pancreas (Rodriguez et al. 1982). The matrix effect of apples on bioavailability of these dihydroxychalcones is highly recommended to have a better insight in the consumption of these constituents as in apples.

Licorice is an important ingredient in traditional medicine and commonly used as a dietary supplement. Thus, the bioavailability and metabolism of this plant, together with its phytoconstituent chalcones, are well studied. One of the studies has been conducted to evaluate the metabolism of three common licorice species, *G. glabra*, *G. uralensis*, and *G. inflata*, in addition to fourteen compounds, including chalcones which were isolated from these plants. This study was conducted by measuring the inhibitory effect of nine cytochrome P450 enzymes using UHPLC-MS/MS cocktail assay. All three licorice species were found to inhibit several drug-metabolizing

cytochrome P450 enzymes at varying degree and showed unique profile of phytoconstituents with potential drug interactions. The unique enzyme interactions profile of licorice species implied the importance of disclosure of botanical species identity and methods of preparation and extraction for the production of dietary supplements. Isoliquiritigenin (**9**) and licochalcone A (**10**) exhibited inhibition of more than 50% at a concentration of 10 μM against CYP2C8 and CYP2C9 genes. Licochalcone A (**10**), which is specific to *G. inflata*, was also a moderate inhibitor of CYP2C19 and CYP3A4 (testosterone) and a weak inhibitor of CYP1A2 (Li et al. 2017).

Isoliquiritigenin (**9**) is found to have poor bioavailability due to its extremely low water solubility. Its oral bioavailability in rats is less than 50%, which greatly diminishes its pharmacological effects (Zhang et al. 2009b, 2013, Qiao et al. 2014). This chalcone is found to conjugate rapidly in the liver and form monoglucuronides by UGT1A1 and UGT1A9 where isoliquiritigenin 4′-*O*-glucuronide is the major conjugate present (Guo et al. 2008). Studies have shown that it is absorbed rapidly and also underwent quick elimination after intravenous and oral administration in mice. The high distribution of isoliquiritigenin (**9**) in the heart, lung, kidney, and liver was observed, indicating that it has good curative effects on these organs. Furthermore, the main route of excretion of this constituent is predicted to be in the kidney where it is detected in high concentration (Qiao et al. 2014). Its high polarity results in its inability to cross the blood-brain barrier (Hua et al. 2010; Li et al. 2008). The diverse pharmacological activities of isoliquiritigenin (**9**) have attracted researchers to study on the enhancement of its absorption characteristics by using nanostructured lipid carrier, which is widely used as an oral delivery carrier for drugs with poor water solubility (Zhang et al. 2014a).

Another chalcone from licorice plant, licochalcone A (**10**) was well studied for its metabolism effects by using human liver microsomes and human hepatocytes in both in vitro and in vivo models. Five oxygenated phase I metabolites were produced by the human liver microsomes, including three major mono-oxygenated metabolites and two minor di-oxygenated metabolites. On the other hand, nine phase II monoglucuronides of licochalcone A (**10**) and phase I metabolites were produced during the incubation with human hepatocytes. One sulphate conjugate and one glutathione conjugate of this chalcone were also formed from human hepatocytes. Furthermore, the enzymes responsible for metabolic biotransformation of licochalcone A (**10**) were identified. The major metabolism of this compound has been predicted to be the phase II conjugation reactions (Huang et al. 2017), even though phase I metabolites are more potent than **10** in terms of pharmacological and toxicity effects. Furthermore, a study was conducted to develop a HPLC method to enable the separation of these chalcones and three of its glucuronic acid conjugates for simultaneous quantification in urine and plasma after intraperitoneal administration of a single dose of licochalcone A (**10**) in rat. The method was found to be selective, reliable, and sensitive and validated according to good laboratory practice (GLP) guidelines (Nadelmann et al. 1997).

The metabolism of cardamonin (**17**), a chalcone present in fingerroot, was studied by using seven major types of P450 enzymes in human and animal liver microsomal

incubation system. The human livers were obtained from autopsy samples (n = 5 Chinese males, 27–48 years old) from Dalian Medical University, China, while the livers of cattle, sheep, and pig were obtained from animals in abattoirs; meanwhile mouse, rat, guinea pig, and dog livers were obtained from the experimental animal center of Shanghai University of Traditional Chinese Medicine, China. HPLC coupled with ion trap MS was utilized for the identification of metabolites. Selective inhibitors were used in this study to inhibit the hydroxylation of this chalcone in order to identify the isozymes involved in the metabolism, and the results showed the isoenzymes are CYP1A2 and CYP2E1. In addition, the outcomes of this study on the species difference on the metabolism of cardamonin (**17**) showed that the guinea pig possesses the metabolic capacity that is the most similar to that of humans and could be used for further pharmacokinetic studies (He et al. 2009). Another chalcone from fingerroot, panduratin A (**19**) was examined for its metabolism effects on activation (phosphorylation) of AMPK and deactivation (phosphorylation) of its substrate acetyl-CoA carboxylase (ACC) in liver (Hep-G2) and muscle (L6 muscle) metabolic cells. The treatment of chalcone increased the activation of p-AMPK and resulted in fat production reduction and energy consumption increment. The treatment also enhanced deactivation of p-ACC, which plays an important role in fat production. The overall results confirmed that panduratin A (**19**) showed anti-obesity effect by facilitating energy metabolism in liver cells (US8653143B2).

9.4 Bioactivities (Animal Aspects)

Biological activities of food or dietary products and its bioactive constituents are widely studied in animal models, which is an in vivo test where the effects are evaluated by using living organisms or cells. The importance of in vivo studies is highly justifiable with the false-positive or misleading results from the in vitro assays, especially in the industry of pharmaceutical and functional foods. Pharmacokinetic aspects are necessary to be taken into account to ensure that the bioactive constituents are delivered to the site of action, as well as the metabolism reactions that are likely to degrade these constituents throughout the journey to the active sites. Thus, the animal model testing is crucial and widely used to further confirm the biological activities obtained from the in vitro models. This section will focus on the in vivo bioactivities of chalcones from the food source.

Naringenin chalcone (**1**) was investigated for its antiallergic activities in the in vivo model due to its abundancy in tomatoes, which are claimed to improve perennial allergic rhinitis. This chalcone was orally administered into mice with airway inflammation, and the results showed that it suppressed the asthmatic symptoms significantly. It worked by inhibiting Th2 cytokine production from CD4 T cells, and mucus hypersecretion was also found to be reduced (Iwamura et al. 2010). In contrast, long-term and high-dose consumption of tomato extract through oral administration did not give any positive effects (Yamakoshi et al. 2004), revealing that naringenin chalcone (**1**) is suggested to be taken as supplement for patients with allergic airway inflammation.

Licochalones, which are present abundantly in licorice plant, are also well-known for their significant anti-inflammatory activities, especially as treatment for allergic airway inflammation. Licochalcone A (**10**) was evaluated for this bioactivity by using an in vivo study on a non-infectious mouse model of asthma. This chalcone was proven to inhibit the production of Th2 cytokines, including IL-4, IL-5, and IL-13 in the bronchoalveolar lavage fluid, and result in a decrease in OVA-specific IgE and IgG of mice serum. It also showed strong inhibition against the OVA-induced eosinophilia in lung tissue and hypersecretion of mucus by goblet cells in the airway, subsequently resolving inflammation (Chu et al. 2013). Similar to naringenin chalcone (**1**), licochalcone A (**10**) is potential to be developed into drugs for allergic airway inflammation because of its ability to delay the progression of inflammation effectively. Also, the anti-inflammatory activities of licochalcone A (**10**) on the inhibition of NO production and PGE_2, as well as the expression of iNOS, COX-2, IL-1β, and IL-6 in LPS-induced RAW264.7 cells, are further confirmed in the animal model. This chalcone is able to suppress the BALB/c mice from LPS-induced endotoxin shock, deducing the ability of **10** in inhibiting the production of inflammatory mediators (Kwon et al. 2008).

Licochalcone A (**10**) also showed antitumor effect in the colon by inhibiting carcinogenesis and metastasis in mouse models. The oral administration of **10** significantly reduced tumor formation in the colon and cell proliferation with expression of cell nuclear antigen, β-catenin, COX-2, and iNOS. It is also found to suppress the level of pro-inflammatory cytokines and chemokines. More interestingly, the survival rate of the mouse is increased by inhibition of liver metastasis and expression of matrix metalloproteinase-9 in the liver. The outcomes suggested that licochalcone A (**10**) is a potential anticancer agent toward colorectal cancer (Kim et al. 2010). Licochalcone B (**11**) also showed antitumor activities both in vitro and in vivo in the tumor model, besides exhibiting positive in vitro effects in the proliferation of malignant bladder cancer cells T24 and EJ. The murine MB49 bladder carcinoma in C57BL/6 mice was used as an in vivo model in this study. Licochalcone B (**11**), which was administered by intratumoral injection every 2 days, showed inhibition in tumor growth at the concentration of 160 μM. The in vivo antiapoptotic effects of **11** were examined, and the results showed microscopic sign of apoptosis. Furthermore, no toxicity was detected in mice serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) at 160 μM licochalcone B (**11**) concentration (Yuan et al. 2014).

In addition, the consumption of licochalcone A (**10**) was also claimed to be able to relieve obesity. In the high-fat diet-induced obesity mouse model, this constituent induces the browning of the subcutaneous adipocytes and successively expresses the key brown adipocyte-specific markers. The finding indicates that **10** plays a role in increasing the thermogenesis, fat oxidation, and lipolysis, besides lipogenesis reduction (Lee et al. 2018). On the other hand, licochalcone A (**10**) exhibited potent in vitro and in vivo activities against *Toxoplasma gondii*, a protozoa which causes toxoplasmosis that possesses serious public health problems. The in vivo evaluation was conducted by using mouse infection model, and the results showed that **10** inhibited the proliferation of *T. gondii* with low toxicity to the HFF host cells. The

survival rate of the infected mice is significantly increased after the administration of chalcone, suggesting that **10** is a promising agent in the treatment of toxoplasmosis (Si et al. 2018).

Isoliquiritigenin (**9**) is another major chalcone from licorice plant that possesses anti-inflammatory effects. A study performed by using mouse model showed that this chalcone showed potent inhibitory effects toward the production of NLRP3 inflammasome-induced IL-1β and activation caspase-1 from hepatic steatosis, white adipose tissue, hypercholesterolemia, adipose tissue inflammation, obesity, and insulin resistance, reflecting its anti-inflammatory activities. The effects shown are even stronger than parthenolide, which is a well-known inhibitor, revealing its potential to be a drug target for the treatment of inflammatory diseases (Honda et al. 2014). This finding was then confirmed by a study on the antidiabetic effect of isoliquiritigenin (**9**), which revealed a blood glucose-lowering and anti-hyperglycemia effect on mice (Gaur et al. 2014). This constituent was also found to inhibit mouse S180 tumors by suppressing GSK-3β, subsequently inducing NF-κB pathway-related protein expressions. TNF-α expression was then increased in NK cells and induced autophagy in S180 tumor cells (Ren et al. 2018). Furthermore, isoliquiritigenin (**9**) was evaluated for its aromatase inhibitor in recombinant protein and MCF-7 cells transfected with CYP19 (MCF-7aro) for the potential to be used as chemoprotective agent against breast cancer for postmenopausal women. Compound **9** was administered as diet for ovariectomized athymic mice transplanted with MCF-7aro cells, and the results showed that it is able to inhibit the aromatase (Ye et al. 2009).

Another chalcone from licorice, licochalone C (**12**) exhibited an important biological cardioprotective function through a range of activities. The study was conducted by using rats which were anesthetized and treated with heparin to prevent coagulation, followed by heart removal for the bioassay. The pretreatment with licochalcone C (**12**) showed significant improvement in the recovery of left ventricular developed pressure (LVDP) and its maximum up/down rate ($\pm$ dp/dt_{max}). The increased levels of superoxide dismutase (SOD) and glutathione/glutathione disulphide (GSH/GSSG) ratio indicates the relieve of oxidative stress. Moreover, this chalcone is able to decrease the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL)-positive cell ratio and morphological changes, revealing its antiapoptotic activities. Licochalcone C (**12**) also exhibited anti-inflammatory effects by relieving the mitochondrial injury and decreasing the levels of creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), and tumor necrosis factor-alpha (TNF-α). The overall outcomes of this study suggest that **12** is a potential therapeutic agent to prevent myocardial ischemia/reperfusion injury (Zhou et al. 2015).

Phloretin (**5**) from apples has been evaluated for antitumor activities on colon, bladder, liver, lung, and breast cancer cells. Its significant antitumor activities were supported by in vivo studies. This chalcone showed inhibition of cell proliferation and arrested cell cycle in human breast cancer cell line MDA-MB-231 in a p53 mutant-dependent manner, besides decreasing the migratory activities of the cells. The in vivo study was done by using BALB/c nude mice bearing MDA-MB-231

tumor xenografts, at which a decrease of *N*-cadherin and vimentin and an increase in p53, p21, and E-cadherin were detected in the tumor (Wu et al. 2018). For inflammation, phloretin (**5**) was shown to suppress inflammatory bowel diseases by inhibiting many pro-inflammatory cytokines in human epithelial cells, besides suppressing TNF-α-induced inflammatory response in human colonic epithelial cells. This animal study was conducted by using trinitrobenzenesulfonic acid (TNBS)-induced rat model, and treatment showed that phloretin (**5**) ameliorated colon inflammation and body weight loss significantly, revealing its potential to be a therapeutic agent for inflammatory bowel diseases (Lee et al. 2011).

Another chalcone from apple, phloridzin (**6**) is a natural food additive that is a competitive inhibitor of sodium glycogen transporter 1 (SGLT1). This causes diarrhea due to the disruption of intestinal brush border resulting from the suppression effect of SGLT1, which is known as a typical symptom of ulcerative colitis (UC). However, an in vivo study showed that constituent **5** could be a dietary supplement of UC by its ability to improve the symptoms of acute colitis, besides protecting the intestinal brush border through promoting the expressions of SGLT1 and series of proteins (Lu et al. 2018). Another study on the hepaprotective effects of phloridzin (**6**) against methotrexate (MTX)-induced hepatotoxicity in terms of histopathological variables, biochemical parameters, and molecular estimation was performed. The rats were administered with MTX to develop liver toxicity and pre-treated with **6**. The outcomes showed that the chalcone significantly reduced hepatic injury through significant relief in oxidative stress, inflammation, and apoptosis in hepatic tissues. The promising results suggest that this chalcone from apple is worthy of further studies in clinical trials for prevention and early treatment of MTX-induced hepatoxicity (Khalifa et al. 2017).

Cardamonin (**17**), a bioactive chalcone present in fingerroot, showed excellent protective effects on liver ischemia reperfusion injury. The study was conducted with the use of a rat model that has been induced using the weight hanging method where the hepatic portal vein, artery, and bile duct have been occluded. Treatment with **17** significantly increased the NO/eNOS expression in ischemia reperfusion injury and decreased the iNOS expression. Thus, it is able to improve functional and structural abnormalities of the liver through relief of oxidative stress and inflammation (Atef et al. 2017). Another study was also found to report the anti-inflammatory effect of cardamonin (**17**) but focused on the intestinal inflammation and mechanism potential in dextran sulphate sodium (DSS)-induced colitis with the use of an animal model. This chalcone was proven to ameliorate mouse body weight, diarrhea, spleen swelling, colon shortening, and histological damage. These effects were observed due to the ability of **17** in reducing the activity of myeloperoxidase and production of NO, TNF-α, and IL-6 in the colon, as well as suppressing TLR4 expression and inactivation of NF-κB and MAPK pathways. The outcomes of this study showed that cardamonin (**17**) is highly potential to be effective in the treatment of human inflammatory bowel disease (IBD) or related complications (Ren et al. 2015).

Cardamonin (**17**) was also proven to show chemoprotective activities in colorectal cancer. The study evaluated this chalcone for its therapeutic effect in the colorectal cancer mouse model which was induced by azoxymethane (AOM).

Cardamonin (**17**) was administered orally at a dose of 10 mg/kg body weight for 16 weeks and showed positive effects against the tumor. The chalcone showed inhibition toward tumor growth, as well as expression of cell cycle arrest and apoptosis in human colorectal cancer cell lines (James et al. 2017). Furthermore, the treatment of cardamonin (**17**) in delayed cerebral vasospasms (DCVS) after subarachnoid haemorrhage (SAH) was evaluated using rat model through internal carotid artery puncture method. The administration of **17** was done through daily intraperitoneal injections at a dose of 7.5 mg/kg body weight. The results showed that the chalcone tends to relieve cerebral vasospasms after SAH and the effects are deduced to be contributed by the inhibitory ability of c-Myc expression and apoptosis, in addition to the expression of α-SMA. Cardamonin (**17**) was also found to inhibit Akt signal transduction pathway by decreasing protein kinase C. The overall results indicate that **17** has the potential to be used to prevent DCVS after SAH (Ma et al. 2018). Interestingly, cardamonin (**17**) also showed a positive effect in the peripheral and central antinociceptive activities using chemical and thermal models of nociception through the involvement of TRPV1, glutamate, and opioid receptors. The administration of **17** by both oral and intraperitoneal routes at doses up to 10 mg/kg body weight gave significant inhibition in abdominal writhing responses that were induced by acetic acid. The results also confirmed that the chalcone exhibited significant analgesic activity in formalin-, capsaicin-, and glutamate-induced paw licking tests but did not give any toxicity effects (Chung et al. 2018).

9.5 Benefits (Human Studies)

Tomatoes have been claimed to possess antiallergic effects and thus led to the interest of researchers to study its effect in the human body. A randomized, double-blind, placebo-controlled human clinical study was carried out on tomato extracts with regard to its histamine release inhibition effect. The extracts were prepared from a seed and skin mixture by using 60% ethyl alcohol at 60 °C for 2 h and confirmed to contain 0.2% of naringenin chalcone (**1**). For the subjects, 33 adults with perennial allergic rhinitis (PAR) comprising of 14 men and 19 women in the age range of 18–56 years were enrolled in this study. The oral administration of tomato extracts was carried out in the form of tablet for 8 weeks with a dose of 360 mg per day. PAR patients did not have any serious adverse effects throughout the study, indicating that the dose given is safe. The outcome of the study demonstrated that the tomato extract is able to improve the nasal symptoms, particularly sneezing, relieving rhinorrhea and nasal obstruction, even though no significant change in the nasal signs was observed by physician. Thus, tomato extracts with naringenin chalcone (**1**) may have therapeutic efficacy in allergic rhinitis (Yoshimura et al. 2007). The results are in good agreement with the in vitro studies on both tomato extracts and naringenin chalcone (**1**) that showed positive effects in antiallergic activities (Kojima et al. 2000).

Apple peel is rich of chalcones, particularly phloretin (**5**) and phloridzin (**6**). A human clinical study was done to evaluate the inhibitory effects of a variety of

botanical extracts on intestinal diacylglyceride acyltransferase 1 (DGAT1) enzyme, including apple peel extract. The enzyme inhibition is associated with attenuation of postprandial hypertriglyceridemia in overweight and obese humans. Four botanical extracts that showed good activities with a primary cell-free DGAT1 enzyme assay were selected to be further studied in a parallel, double-blind, placebo-controlled clinical trial. Apple peel extract which contains 5% of phloridzin (**6**) was selected as one of the extracts. Ninety healthy, overweight, and obese subjects were administered with 2 g of extracts for 7 days, and their serum triglyceride (TG) levels were measured before and after consuming high-fat meal. The results showed that the apple peel extract did not show a significant reduction in TG level, even though it showed relatively good inhibition in the in vitro assay. Instead, whole grape extract was the only extract found to have potential to inhibit DGAT1 enzyme without intolerable side effects (Velliquette et al. 2015).

For licochalcone A (**10**), human clinical studies are mainly focused on the topical formulation for skin diseases. A double-blind, randomized, vehicle-controlled human clinical study was carried out to evaluate skin tolerability and efficacy of moisturizer containing licochalcone A **(10)**, l-carnitine, and 1,2-decanediol (active formulation) for acne treatment. One hundred and twenty Asian subjects were recruited for a 8-week study and subjects were given adapalene gel and the combination of active formulation and adapalene gel for the comparison effects on the severity of acne, skin bio-engineering measurements and skin tolerability. The results showed that the active formulation demonstrated significant relief in inflammatory lesions and total lesions at the end of the study without flare-up, as well as less skin irritation when compared to other groups. Thus, the moisturizer containing the licochalcone A (**10**), *l*-carnitine, and 1,2-decanediol is able to prevent cutaneous irritation and further enhance patients' adherence to acne medications (Chularojanamontri et al. 2016). Furthermore, another human clinical study was reported to focus on anti-irritative efficacy of a cosmetic formulation containing licochalcone A (**10**). The chalcone exhibited significant reduction in erythema in post-shaving skin irritation model, as well as UV-induced erythema formation, suggesting the therapeutic skin care benefits of licochalcone A (**10**) on sensitive or irritated skin (Kolbe et al. 2006).

A variety of products which contain fingerroot extracts are available in the market. The consumption safety of the extract was evaluated in human clinical studies, and no adverse effects were reported for these products. A human clinical study on the photoaging properties of *Boesenbergia pandurata* has been conducted by measuring skin hydration, gloss, wrinkling, and elasticity. The study was carried out with the use of a double-blind method on the ethanol extract of the fingerroot plant, which contains 8% of panduratin A (**19**). The outcomes of this clinical study by using 92 subjects showed that consumption of the extract powder in tablet form showed significant enhancement in skin hydration and gloss, as well as reduced wrinkling after 12 weeks. The tablet is made up of sodium caseinate (63 mg), dextrin (87 mg), and extract (150 mg). In terms of skin elasticity, no significant difference was observed. More importantly, no adverse symptoms were observed throughout the study period. Thus, the extract of fingerroot that contains the bioactive chalcone

has potential to be used as nutraceutical or nutri-cosmetic material for human skin (Kim et al. 2017).

9.6 Application in Food

9.6.1 Tomato

The tomato is a common food known to have health benefits due to its cholesterol-free and low-fat properties. Moreover, it is highly nutritious with the presence of vitamins, carotenoids, and phenolic compounds such as flavonols and chalcones, together with their conjugates. The consumption of tomatoes is growing extensively, as well as the demand for tomato-based products. Common examples of the products are ketchups, tomato juices, and tomato paste, which are used as food ingredients. The processes of these products include washing, dehydration, hydration, cooking, cleaning, and packaging. The in vivo bioavailability of phenolic compounds, especially flavonols and chalcones, in these products plays an important role in their accessibility and extractability from food (Bugianesi et al. 2004; Simonetti et al. 2005). Thus, studies have been done on the phenolic fingerprint for tomato-based products.

The presence of the types of phenolic compounds is greatly depending on the varieties of tomatoes and cultivation conditions, including light, temperature, fertilization and irrigation (Slimestad and Verheul 2005). Naringenin chalcone (**1**), a major chalcone found in tomatoes, is easily degraded to its isomeric flavone, naringenin (**21**) during the processes of making tomato-based products from the fresh tomatoes. Both of these structures were presented in Fig. 7. The degradation is due to the spontaneous cyclization of chalcone during the entire process (Krause and Galensa 1992; Capanoglu et al. 2008). Tomato paste is a common tomato-based product, and its industry processes involve fresh tomatoes chopped in a "breaker" unit and heating of the pulp after the seed and skin are removed, followed by the evaporation process to remove the moisture and finally pasteurization for packaging as shown in Fig. 8 (Capanoglu et al. 2008). The physiological and biochemical processes which occur during these processes are important to maintain or improve the nutritional value of tomato pastes, in terms of phenolic compounds which are chalcones that we referred to in this case. Naringenin chalcones (**1**), which are functional ingredients in the food industry (Peschel et al. 2006), undergo degradation into its isomeric flavone, naringenin, upon the production of paste. Studies showed that the "breaking" process is the most critical due to the significant increase in the amount of chalcones but with a sudden drop after pulping with the removal of seed and skin (Capanoglu et al. 2008). Thus, the recovery of chalcones from the seed and skin of tomatoes is suggested to be done firstly by extraction and return these chalcones into the paste in order to enhance the health benefits of the tomato paste.

Other tomato-based products such as ketchup and tomato juice are considered ambient stable products. The technological process to produce ketchup involves mixing the pasteurized tomato paste with sugar, salt, vinegar, spices, and

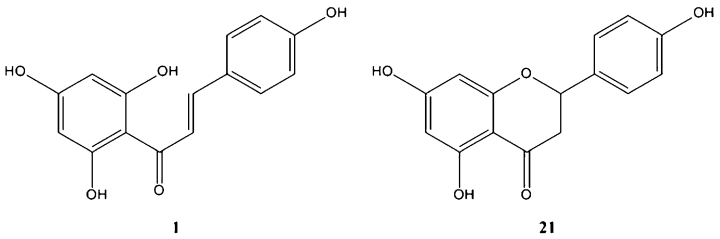


Fig. 7 Structures of naringenin chalcone (**1**) and naringenin (**21**)

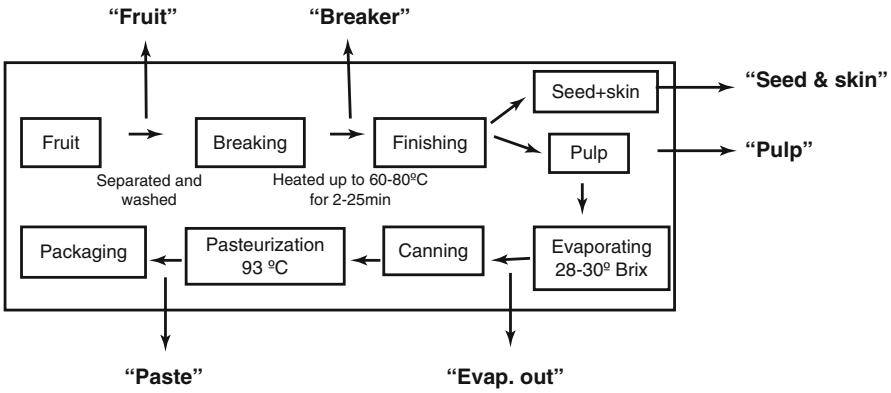


Fig. 8 Industry processes of tomato-based products (Capanoglu et al. 2008)

preservatives, followed by pasteurization at 96 °C for 4–6 min and packaging. The tomato juice is obtained by extraction and concentration and formulated with the addition of extra virgin olive oil, citrus, and salt. It is then pasteurized at 80 °C for 20 min and bottled. Phloretin-*C*-diglycoside (**3**) and dihydroxy-dimethoxychalcone-*C*-diglycoside (**4**) were detected in ketchup and tomato juices with only the former constituent (**3**) found to be present in fresh tomato previously (Vallverdú-Queralt et al. 2011). The production of **4** is deduced to be the happened during the industrial processes where technological practices are utilized such as dehydrations and stabilization (Gahler et al. 2003; Galicia-Cabrera 2007). The main challenge in the production of these products is to maintain the consistency of the physicochemical parameters by choosing the right variety of tomatoes and their ingredients.

9.6.2 Apple

Cider is a popular drink which is an alcoholic beverage made from fermented apple juice. Apple cider is easy to make, and its primary contents are water, ethanol, sugars, organic acids, and phenolic and aromatic compounds. The most important content of cider is the phenolic compounds due their major contribution toward the

organoleptic characteristics such as flavor, color, bitterness, and astringency (Alonso-Salces et al. 2006). The composition of organic acids used in the fermentation process is another important indicator for the quality of cider. In addition, amino acid content is a crucial factor in the fermentation process because they are the nutrients for the yeast. Thus, changes in phenolic compounds and organic acid and/or amino acid content are important when monitoring the fermentation process and assessing quality control of the final product. As mentioned, apple is a rich source of anthocyanins, dihydrochalcones, and flavonols, as well as hydroxycinnamates. On top of these metabolites, tyrosol is formed from tyrosine by yeasts. Metabolites profiling studies were conducted during fermentation process for the production of cider to monitor the changes on metabolites, and as the comparison profile with fresh apple and apple juice.

A higher amount of chalcones are usually detected in the cores and seeds of the apples; thus these constituents are being discarded if fresh apples are consumed. Conversely, apple products such as juices and ciders are produced by using whole fruit, indicating that the intake of chalcones will be higher if these products were consumed instead of fresh fruits (Tomás-Barberán and Clifford 2000). Studies showed that the amount of phloridzin (**6**) in apples varies greatly depending on the cultivar with an amount ranging from 5 to 10 mg/kg present in most of the cultivars. For the examples of apples with bigger range of amount, Kemerrien (French) cider apples contain 169 mg/kg of phloridzin (**6**), English cultivars contain 190 mg/kg, while Verde Doncella contains less than 0.1 mg/kg (Burda et al. 1998; Amiot et al. 1992; McRae et al. 1990). The difference in the amount of **6** in the apple results in different amount of **6** extracted when these cultivars are processed into juice or cider.

Apple pomace has the highest amount of phloridzin (**6**) if compared to the flesh and skin of the apple. Other chalcones, phloretin 2′-*O*-xylosylglucoside (**7**) and 3-hydroxyphloridzin (**8**) with a higher polarity than **6**, were detected in the pomace (Lu and Foo 1997; Burda et al. 1998). These chalcones are another major constituent in apple and used as marker compounds for apple-based products previously (Spanos and Wrolstad 1992; Lea and Arnold 1978). Besides, two derivatives of phloretin (**5**) were detected as the minor constituents and deduced to be the di-glycosides of phloretin (**5**) due to their medium polarity properties (Chinnici et al. 2004). Studies showed that apple pomace has a high level of polyphenols, including chalcones that are valuable in the food industry.

In addition, the total content of dihydrochalcones in the commercial apple juice, regardless of clear or cloudy in appearance, is speculated to be 5–10 times higher than that of domestic juice obtained from extractors (Versari et al. 1997). This is because the whole fruit that is used in the industrial process undergo thermal treatment which in turn inactivates the enzymes that degrade the dihydrochalcones. The pasteurization process which involves heating at 105 °C for 45 min led to a higher rate of degradation of phloretin conjugates if compared to sterilization which is conducted at 120 °C for 20 s. Pasteurization, which involves a higher temperature, will inactivate the oxidative enzymes. On the other hand, filtration process in the clarification of apple juice will decrease the amount of dihydrochalcones such as phloridzin (**6**), even more significant if the filtration time is prolonged due to the

retention on the filters. Commercial pectinases, which are usually used in apple juice clarification, were found to decrease the content of dihydrochalcone intensely by approximately 90% (Mangas et al. 1997). Furthermore, the content of phloretin conjugates present in the apple juice degraded drastically associated with the processes involving enzymatic treatment, indicating its high specificity toward pectinases (Di Lecce et al. 2013).

9.6.3 Licorice

There are approximately 20 known species of licorice (*Glycyrrhiza*), and half of them are used as pharmaceutical ingredients in herbal remedies by many countries around the world. This plant is usually used in herbal medicines as dry raw slices, roasted slices, or slices baked with honey. Different ways to use the licorice will definitely lead to different chemical composition in the plant, including chalcones. Studies have shown that raw licorice possesses higher anti-inflammatory activities than roasted licorice. Some studies have been done to examine the roasted licorice root under a different condition, in the form of solvent extracts. The major constituents of licorice are glycyrrhizin and a pair of isomers, liquiritigenin (**22**) and isoliquiritigenin (**9**) with the structures shown in Fig. 9. The isomers are biosynthetically related and interchangeable. The conversion can occur in a function of time and temperature to reach an equilibrium of 9:1 proportion. The isomerization of isoliquiritigenin (**9**) to liquiritigenin (**22**) was very likely to occur during the processes of licorice, from drying to storage and finally preparation of a boiling alcoholic extract (Simmler et al. 2013). These constituents are claimed to be coexisting in licorice plants. One of the studies which was conducted by extracting roasted licorice root with 95% ethanol showed that the amount of isoliquiritigenin (**9**) detected is lower than both the dry roasted and honey roasted licorice root ethanolic extracts refluxed at 200 °C and 250 °C for 30 min. Among the extracts, the highest amount of **9** was detected in the honey roasted extract refluxed at 250 °C (Sung and Li 2004).

Licochalcone A (**10**) and licochalcone C (**12**) were found to be useful as food preservatives due to their excellent antibacterial activities, suggesting their potential use in the food industry to prevent food spoilage and act against pathogenic bacteria. These chalcones are active against bacterial strains of *Bacillus cereus*, *Bacillus subtilis*, and *Clostridium sporogenes* (Haraguchi et al. 1998; Tsukiyama et al.

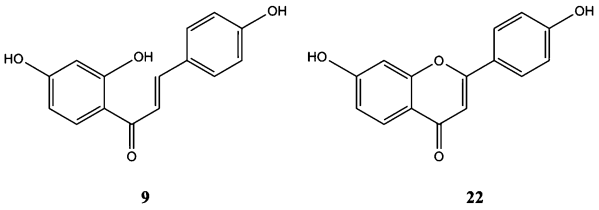


Fig. 9 Structures of isoliquiritigenin (**9**) and liquiritigenin (**22**)

2002), attributed to the prenyl side chain that increases the hydrophobicity of the whole molecules and in turn allows them to penetrate into the cell membrane and act against the bacteria. Licochalcone A (**10**), which presents as the major constituent in many licorice plants, showed positive effects against Gram-positive bacteria such as *Enterococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Staphylococcus* spp., *Streptococcus* spp., and especially *Bacillus* spp. with MIC values of as low as 2–3 μg/mL. Its antibacterial activities are stronger than isoliquiritigenin (**9**). Interestingly, this chalcone is highly resistant to a variety of condition, including temperature of up to 121 °C for 15 min, pH 5.0–7.0, 3% (wt/vol), NaCl (salt), and 20 μg/mL of protease digestion (Tsukiyama et al. 2002). Compound **10** was also found to completely inhibit the outgrowth of *B. subtilis* spores. The outcomes prove that licochalcone A (**10**) is a highly potential compound to be developed as antibacterial agent or preservative in the food industry, particularly soup and fermented foods that contain high level of salt and protease, respectively.

9.6.4 Fingerroot

Fingerroot, which is rich of chalcones, has been widely used in culinary as spices or herbs, especially in local Thai food. The foods are usually served raw or cooked by different methods such as frying, boiling, and grilling. Examples of food include curry and soup, which is a combination of herbs and vegetables with fingerroot as one of the common herbs. Due to the high number of herbs used in culinary, Thai food are considered as health beneficial food. The health benefits of certain cooked food were studied and proven to exhibit antioxidant and anti-mutagenic activities (Nakahara and Trakoontivakorn 1999; Nakahara et al. 2002). Interestingly, one of these studies showed that thermal sterilization that requires high heating temperature has enhanced both the mentioned activities, suggesting that heat treatment is important in improving the beneficial effects of certain food (Tangkanakul et al. 2011).

The antimicrobial and antifungal properties of fingerroot suggest that this herb could be used as food preservative or food packaging, apart from being a natural antioxidant. Natural food preservative from plant extracts is always in high demand due to the increasing number of food poisoning cases and illness resulting from artificial food preservatives (Shan et al. 2007). The crude extracts of fingerroot were found to exhibit good antibacterial activities, especially against antibiotic-resistant strains. These activities were suggested to be contributed by a combination of mechanism of actions due to the presence of different constituents, including cell wall degradation, cytoplasmic membrane and membrane proteins deconstruction, intracellular content leakage, and cytoplasm coagulation (Khan et al. 2009; Burt 2004).

The choices of extraction method of fingerroot should be taken into serious consideration in order to make sure that the important constituents, including chalcones, will not be degraded after the extraction process. Studies have shown that ultrasound-assisted extraction (UAE) caused a drop in the antimicrobial activity of the extract (Thongson et al. 2004), even though it is widely applied in the food

industry due to its significant improve in the extraction yield, shorter extraction time, and lower amount of organic solvent used if compared to conventional extraction methods (Dolatowski et al. 2007; Vilkhu et al. 2008). However, the temperature control in UAE to achieve mild extraction condition may result in an enhanced amount of the constituents, especially chalcones which contribute to the bioactivities of the extracts. Furthermore, solvent selection is a crucial decision to extract the constituents in the highest amount, besides considering the safety and environmental issues. Ethanol is the solvent that has been recommended and widely used in herbal medicine manufacturers due to safety issues and ethanol extracts are usually showing excellent anti-microbial activities against both Gram positive and Gram negatives bacterial strains (Yi and Wetzstein 2011).

4-Hydroxypanduratin (**20**), which is present in fingerroot, was found to possess a variety of biological activities such as antioxidative, anti-mutagenic, antivirus, and antibacterial activities (Trakoontivakorn et al. 2001; Shindo et al. 2006; Cheenpracha et al. 2006). For antibacterial activities, it is found to be strongly bioactive against many foodborne pathogens including *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Listeria monocytogenes*, *Proteus mirabilis*, *Vibrio parahaemolyticus*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (Marliyana et al. 2015, 2017). Similar to the chalcones from licorice plants, 4-hydroxypanduratin (**20**) has high potential to be used as a natural food preservative.

9.7 Safety: Toxicity and Side Effects

Food sources rich in chalcones are apples, tomatoes, licorice, and fingerroot. These foods are common and consumed in raw or processed forms and used as food ingredients, food additives, and nutritional supplements. Hence, it is crucial to examine the toxicity and/or side effects of these food. Fresh food samples and extracts, together with their major constituents, particularly chalcones, are usually evaluated. Safety evaluations of the food and drugs for human consumption can be carried out by animal models to examine their toxicity and predict their safe doses for humans. The toxicity effects examined in the animals include hematological, gastrointestinal, and cardiovascular adverse effects (Olson et al. 2000).

9.7.1 Apple

Apple extracts are known to have strong antioxidant effects and other physiological functions. The safety tests of the consumption of the extracts were carried out on some of the market products of apple polyphenols, including phloretin (**5**) and phloridzin (**6**) with their conjugates. One of the findings on the polyphenol extracted from unripen apples revealed that the product is safe to be consumed at an average dietary dosage level, even though it was found to possess mutagenic effect at a dose of 120-fold higher than the estimated average dose. The unripen apple extract does not show any significant hematological, clinical, chemical, hispathological, and

urinary effect at high dosage through acute and sub-chronic oral toxicity tests (Shoji et al. 2004; Fujiwara et al. 2013). Another apple polyphenol extract obtained from matured apple fruits was examined for its genotoxicity. Phloridzin (**6**) is detected as one of the polyphenols in the extract. The results confirmed that the extract does not show any genotoxicity at a 100-fold safety margin through hepatocytes unscheduled DNA synthesis (UDS) and bone marrow micronucleus and comet assay in intestinal cells, thus suggesting that the daily intake is acceptable (Lina et al. 2012).

Conversely, studies showed that the consumption of fruits is associated with cardiovascular and other chronic diseases, even though the effect is not significant. One of the studies was done to examine the protective effects of the fresh apple extracts on human umbilical vein endothelial cells (HUVEC), which were exposed to cytotoxic glycated protein (GFBS)/iron ($FeCl_3$) chelate. The findings showed that the extracts have cells recovery ability, suggesting its protective effect in the patient with diabetic angiopathy (Nishigaki et al. 2010). Furthermore, the apple peel extracts were proven to possess protective effect against cardiac toxicity that is induced by arsenic trioxide (ATO), which is an effective drug for the treatment of acute promyelocytic leukemia (APL). The protective effect of the peel was proposed to be attributed to its well-known antioxidant properties. Thus, the peel has the potential to be used as a nutraceutical for cancer patients on ATO therapy. Dihydrochalcones, particularly phloretin (**5**) and phloridzin (**6**) are the main ingredients of the peel that contributed to these protective effects during the intake of the apple peels (Vineetha et al. 2014).

9.7.2 Tomato

Similar to apple, tomatoes were proven scientifically to be beneficial in the prevention and therapy of toxicity induced by pharmaceutical drugs. For examples, a study showed that tomato paste possessed in vivo protective effect against the damage resulted by acetaminophen and amiodarone. The protective effects shown were against oxidative stress, tissue damage, and biochemical indices against acetaminophen-induced acute hepatotoxicity, as well as lower effect against amiodarone-induced lung toxicity and little effect against cyclosporine A-induced nephrotoxicity (Jamshidzadeh et al. 2008). Nevertheless, a human clinical trial study showed that the subjects who consumed the apple peel extract at a dose of 360 mg per day for 8 weeks did not show any adverse effect throughout the study, and no significant deviation was observed in the results of urinalysis, blood, and biochemistry of the subjects. The results indicated that the apple peel extract, which contains 0.2% of naringenin chalcone (**1**), is safe for consumption (Yoshimura et al. 2007).

However, some studies showed that the consumption of tomato and/or its products may cause harmful effects. The common harmful effects are allergies, gastroesophageal reflux disease (GERD) or heartburn, kidney problems, irritable bowel syndrome (IBS), lycopenodermia, urinary problems, body aches, arthritis, and migraine. For allergies, consumption of raw tomatoes may cause allergies with different symptoms such as sneezing, itchy throat, face swelling, and dermatitis, as

well as lead to extremely severe anaphylaxis in children or individuals who are susceptible to allergies (Chow and Sediva 2013; Zukiewicz-Sobczak et al. 2013; Gann et al. 1999). The allergy responses differ based on the composition of compounds present in tomatoes. The identified allergens are β-fructofuranosidase, profilin, superoxide dismutase, pectinesterase, polygalacturonase, cyclophilin, lipid transfer proteins, Lyc e 2, and Lyc e 3 (Sandra et al. 2003; Westphal et al. 2004; Foetisch et al. 2001; Kondo et al. 2001; Asero et al. 2000; Quynh et al. 2006; Welter 2014) and none of these allergens belong to chalcone. Thus, there is no evidence that chalcones from tomatoes will give these allergic responses. Furthermore, patients with GERD were found to have higher consumption frequency and amount of tomato and tomato juice (Jarosz and Taraszewska 2014; Wang et al. 2004). The effects were claimed to be attributed to organic acids and citric and malic acids but not chalcones (Priyadharsini and Muthukumar 2016).

Even though some research outcomes have shown that regular consumption of tomatoes may have protection effect against kidney problems, the US Department of Health and Human Services advices the reduced intake of tomatoes for patients with kidney problems due to its high potassium content and the presence of oxalate that will result in hyperkalemia and formation of kidney stone (Massey et al. 1993; Siener et al. 2016; Dogukan et al. 2011; Phillips and Polzin 1998). An excessive intake of lycopene, as a result of high tomato consumption, can lead to lycopenodermia, a condition which involves discoloration of the skin. Glycoalkaloids has been claimed to contribute to gastrointestinal tract disorders and arteritis pain as a result of consumption of green tomatoes. Moreover, urinary problems such as urinary incontinence and cystitis and undesired bladder symptoms are associated with consumption of tomato and its products (Townsend et al. 2013; Friedlander et al. 2012). These negative effects were not related to chalcones present in tomatoes.

9.7.3 Licorice

Licorice is claimed as a valuable medicinal plant and widely used as traditional medicine in many countries. Due to its pharmacological activities, this plant has been reported to possess many side effects. The adverse effects are observed after the continuous consumption of significant amount of licorice (up to 100 g/day) for several years. One of the common side effects found with long-term ingestion of licorice is blood pressure elevation, which is also known as hypertension. The side effect was found to be closely related to the signaling pathway of renin-angiotensin-aldosterone system. The phytoconstituents of licorice induce aldosterone action in the kidneys while binding to mineralocorticoid receptors, resulting in hyper-mineralocorticoid syndrome. In addition, hypokalemia (low potassium level) and hypernatremia (high sodium level) are commonly found as side effects that lead to edema subsequently. These side effects may result in a more severe condition that induces myopathy. The onset and level of severity of these side effects greatly depend on the dosage level and duration of licorice ingestion, as well as individual susceptibility

(Zadeh et al. 2013). For example, subjects with delayed gastrointestinal transit time are prone to these side effects due to enterohepatic cycling and reabsorption of the metabolites of licorice.

Another side effect of licorice plants is ocular side effects with some transient visual loss cases reported due to the consumption of licorice. The visual symptoms reported were similar to that of ocular migraine but without headache. The side effect was found to be associated with vasospasm of the brain and retinal or optic nerve blood vessels. Clinical studies showed that the ocular side effects occured when consumption of licorice is high. Thus, intake of this plant should be in caution, especially for those who have migraine headache, to prevent level-up effects (Jun and Zhengang 1988; Walker et al. 1992, 1994).

Licochalone A (**10**), which is a major constituent of licorice, was studied for its toxicity effect with the animal models. The chalcone showed nontoxicity up to 2000 mg/kg body weight through oral administration in the anti-inflammatory and antitumor tests of licorice plants and licochalcone A (**10**) (Kim et al. 2010). Moreover, the administration of licochalcone A (**10**) at 1 mg/kg body weight through oral gavage technique in mice did not detect any induction of nephrotoxicity, hepatotoxicity, and oxidative stress. This study was done to investigate its inhibitory effect toward solid tumors in CT-26 cell-inoculated mice. In addition, the administration of **10** prior to cisplatin treatment resulted in a preventive effect on the cisplatin-mediated increased level of serum nitric oxide (NO) and tissue lipid peroxidation, subsequently recovering the depleted reduced glutathione levels in the tissues. Thus, licochalcone A (**10**) is beneficial in reducing the side effects of cisplatin therapy in cancer patients, instead of contributing to the toxicity or side effects (Lee et al. 2008). Moreover, compound **10** showed low toxicity against the human foreskin fibroblast (HFF) host cells through MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide] test besides the evaluation of the antiproliferative effects toward *Toxoplasma gondii*, which results in toxoplasmosis (Si et al. 2018).

9.7.4 Fingerroot

The acute toxicity of the fingerroot extract was studied in conjunction with its wound healing activities in animal model. The outcome of the study showed that oral administration of the extract up to a dose of 5 g/kg body weight is safe and no drug-related toxicity was detected. Clinical observations, as well as the data collected from hematology and hispathology showed no significant difference with the control data (Mahmood et al. 2010). The flavanones, which have the closest structures with chalcones, from fingerroot extracts were examined for their toxicity and mutagenicity in the rats. Pinocembrin (**23**) and pinostrobin (**24**), as shown in Fig. 10, were administered orally at the dose of 500 mg/kg body weight, and no death was observed. The weights of the body and vital organs, as well as blood biochemistry values obtained for flavanone treated rats, were comparable with the control. Moreover, mutagenicity test indicated that no micronucleus formation or mitotic index was encountered with dose up to 100 mg/kg, suggesting non-mutagenic

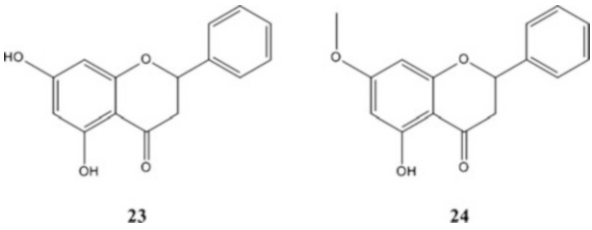


Fig. 10 Structures of pinocembrin (**23**) and pinostrobin (**24**)

properties of the two compounds (Charoensin et al. 2010). Similarly, one of the market products of the fingerroot extract powder reported no mutagenicity or genotoxicity, and acute oral toxicity showed that the LD_{50} value is greater than 2,000 mg/kg body weight in male and female rats.

Similar to other chalcones present in the food, panduratin A (**19**) possessed hepaprotective effect instead of toxicity effect. An animal study was conducted with this chalcone, which was isolated from the rhizomes of *Boesenbergia rotunda*. The compound was administered for 8 weeks by oral gavage at several dosage levels up to 50 mg/kg body weight into rats with liver damage induced by intraperitoneal injection of thioacetamide. Acute toxicity was assessed, and a safe dose of up to 250 mg/kg body weight was confirmed. The study revealed that **19** could reduce platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β1), as well as hepatic metalloproteinase enzyme (MMP-2) and its inhibitor extracellular matrix protein (TIMP-1), suggesting that panduratin A (**19**) is a potential compound to protect the liver from the progression of cirrhosis (Salama et al. 2018).

A study has been done on the structure-toxicity relationship of the eighteen chalcones, including cardamonin (**17**) and pinostrobin chalcone (**18**) which are present in fingerroot plants. Multiple cell-based assays were conducted in the study to evaluate different aspects of toxicity such as cell proliferation, mitochondrial health, cell cycle, and other cellular features by using a human hepatic stellate cell line. The outcome of the chalcone structure-based toxicity model is presented in Fig. 11. The study revealed the active structural element and moieties that are deduced to contribute to the toxicity in the cells. Pinostrobin chalcone (**18**) which has a hydroxyl group at position R1 (refer to Fig. 11) did not show any toxicity effect, while the substitution effect in the rings showed that substitution at ring A gave a higher impact than that of ring B. The study is claimed to be important to understand the molecular mechanism of pharmacological effects, as well as side effect (Zenger et al. 2015).

9.8 Marketed Products

A variety of apple products can be found in the market, including the polyphenol extracts. The current market of apple polyphenol is small, but it is growing significantly due to market demand on global health and nutrition. The products from

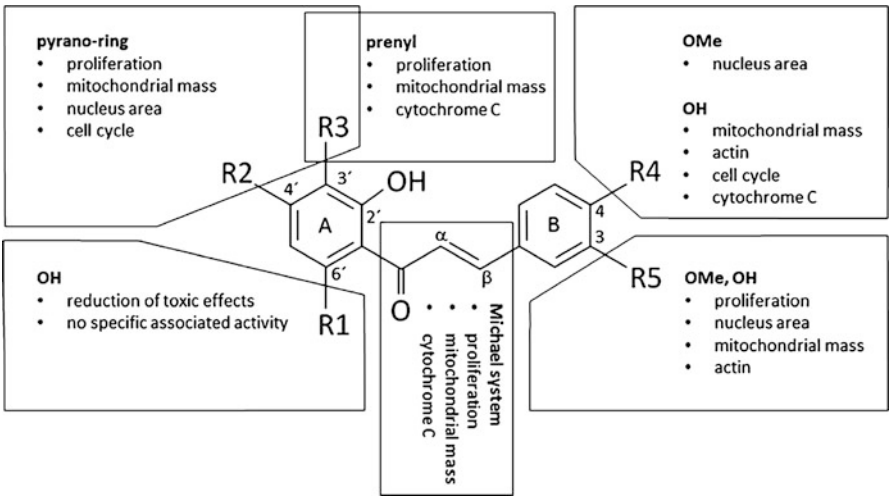


Fig. 11 Significant structure-toxicity relationships of distinct substitutions at chalcone (Zenger et al. 2015)

apple polyphenol extract differ in composition, depending on the type and maturity of the apples used. These products are claimed to be high in various physiological activities, especially antioxidant activities. The examples of the products are Applephenon® and Evesse™. Applephenon® is a patented product with polyphenols extracted from wild and unripe apples produced in Central Asia. The consumption safety of this product was supported with scientific data, and it is safe to be consumed at average dietary level (Shoji et al. 2004). Its antioxidant activities were confirmed by oxygen radical absorbance capacity (ORAC) value which is extremely high in comparison to other polyphenol products. Chalcone is one of the polyphenol compounds present in the extract.

On the other hand, Evesse™ is a product of apple polyphenols extracted from the apples. The whole fresh apples are de-waxed by ethanol and then combined with freeze-dried apple granules and hot water. After that, the enzymes are added for denaturation purpose in order to yield sugar-rich syrup while the residual carbohydrate polymers are degraded with enzymes to remove sugar. Evesse™ is precipitated with food grade ethanol and consists high amount of polyphenol with phloridzin (**6**) at a concentration lower than 5%. This product is available in powder and granules and is tasteless and odorless, allowing easy formulation in the food industry. The market of the product is focused in the USA and Europe in terms of foods and beverages, even though it was initially targeted as a supplement. Safety evaluation test on Evesse™ EPC showed that it has an adverse effect in in vivo studies of unscheduled DNA synthesis (UDS) test in hepatocytes, bone marrow micronucleus test, and comet assay in intestinal cells. Nevertheless, this product showed a positive effect in both in vitro bacterial reverse mutation and mouse lymphoma tests (Lina et al. 2012).

Licorice is widely found as one of the active ingredients in food products, dietary supplements, and traditional medicine products. It has been proven to be active toward a wide range of ailments (Omar et al. 2012). Commercially, licorice exists in two principal forms which are root and extract (Isbrucker and Burdock 2006). Some of the examples of products containing licorice in the market are bronchial wellness tea, cleanse and detox tea, throat spray, energy vitality drink, fresh breath spray, and digestive tea. This plant contains a number of chalones, which are bioactive phytocomponents responsible for the claimed health benefits.

Fingerroot products in the market come in essential oil, powder, and dried sliced forms. Among the products, essential oil is widely seen and known for its warm and camphorous aroma. One of the examples is Pandoradiet™ with panduratin A (**19**) as a marker compound. The content of compound **19** should not be less than 3.52%. The product has been claimed to have high water solubility and stability toward heat and low pH, resulting in various types of food application such as drink, capsule, and tablet. The products of fingerroot have been claimed to have therapeutic effects which include healthy digestive system support and calming effects.

9.9 Patents

9.9.1 Apple

A number of patents related to apple and its chalcones, phloretin (**5**) and phloridzin (**6**), are available. Some of these inventions are focused on the purification of chalcones from apples. One of the inventions disclosed the method to obtain the highly pure phloridzin (**6**) from apple tree branches. The method involves the pretreatment steps of raw material, extraction using CO_2-enzyme supercritical method and purification with centrifugal partition chromatography (CN105777822A). The method was claimed to be safe and highly effective with simplified preparation processes, in addition to shorter purification time and higher yield. Another invention focused on the purification of **6** from apple root bark. This invention (CN102643315B) filed the a series of purification methods include the extraction of raw materials by pulverization, filtration of extracts using acid water, precipitation by centrifuging the supernatant and recrystallization using purified water to obtain pure phloridzin (**6**). The inventors claimed that the method is applicable in industry scale due to its simplicity, low cost, and low polluting effect. For apple pomace, a patent (CN103214530B) was found on the purification for phloridzin (**6**). The steps include crude extraction using ethanol in a range of ratio values in thermostatic water bath, adsorption purification with microporous resin by pH adjustment, and elution of pure phloridzin (**6**) with pH-adjusted ethanol.

The applications of phloridzin (**6**) in food, cosmeceutical, and nutraceutical industries are widely recorded as well. One of the patents found is regarding the best composition of phloretin (**5**) or phloridzin (**6**) to be used the food products, food additives, supplements, animal feeds and pharmaceutical preparations for their beneficial effects (DE10053496A1). The concentration of compounds **5** and **6** in the

food products is preferred to be in the range of 1–100 μM for better absorption characteristics in the small intestine. Another invention was found on the application of phloridzin (**6**) in terms of the preparation of a hepatic or healthcare food. This invention (CN101683351B) provides a pharmaceutical composition of **6** in a general formula that gives a pure natural preparation which is safe and reliable with low toxicity and no adverse effect. The medicament can be used in the form of tea which has a delicious taste and possesses protective effect against hepatic injury, as well as treating acute alcoholism by improving intoxication such as headache, dizziness, and hypodynamia. Besides, the process of obtaining the phenolic fraction of fruits from the Rosaceae family which is rich in phloretin (**5**) and phloridzin (**6**) were filed in a patent (US7427418B2). These phenolic fractions are claimed to be beneficial to be used as the cosmectical and dietary or nutraceutical agents. The cosmetic product is made up of an antioxidant composition of a phenolic fraction, which consists of more than 20% by weight of polyphenols with at least 10% of phloridzin (**6**).

9.9.2 Tomato

Tomato products are usually present in processed form preserved in bottles and cans. Examples are tomato juice, puree, ketchup, chutney, and pickles, and these products are ready to serve, which are highly savored by consumers. A patent (US3549384A) has been filed on the preparation method of tomato products, specifically to prepare an improved tomato ketchup from fresh whole tomatoes. This invention suggested that the conventional method of tomato ketchup manufacture results in loss of flavor and higher tendency of tomato ketchup separation during storage. Thus, the method to improve the production of tomato ketchup was reported. The method includes breaking the tomatoes into particulate solids and heating the surface within seconds to a temperature that is able to inactivate the pectinase enzyme, subsequently heating the ketchup mixture completely to break down the enzyme in order to minimize enzyme activities. Besides, another patent (US2858226A) was filed for the dehydration process of tomato juice to obtain the solid dried products, which are highly nutritious. A previously used puff-drying technique is not guaranteed with good results because the juice concentration does not expand properly during hydration, thus results in failure of dehydration and impossibility of rehydrate. Therefore, this invention was focused on the dehydration process where the products obtained are in free-flowing crystalline granular particles that will not coalesce and gum even exposed to the air for a long period of time. More importantly, the desired characteristics of the products have been retained where the flavor and nutritional value of the products are similar to the freshly prepared juice. An old patent (US2092729A) concerning the preparation of tomato juice beverage was filed to obtain the juice with adequate vitamin content. Briefly, the method includes the submerging of fresh tomato in a hot pool with sterilizing temperature to obtain pulp and juice, followed by extracting the pool of juice and entrapped pulp, supplemented by the condensate available from the additionally heat-treated pulp and entrapped juice.

Naringenin chalcone (**1**), which is present in tomatoes, has been found to possess a wide range of biological activities. Thus, there are a few patents filed for this chalcone on its pharmacological effects. One of which is focused on the production of tomatoes which are rich in naringenin chalcone (**1**) and have excellent taste. This invention (JP2011078334A) reported that genetic recombination method which was previously used to increase the content of **1** in the mature phase of tomatoes possesses adverse effects in human body. In this report, the tomatoes are produced by breeding selection without using genetic recombination techniques and have high content of **1** in fully ripe stage (>10 mg). The antiallergic activities of this chalcone was also filed in an invention. This invention (JP2002080352A) is focused on naringenin chalcone (**1**) as an active ingredient in an antiallergic agent. This antiallergic agent is able to prevent or treat allergic diseases, including anti-inflammatory disease, atopic dermatitis, allergic rhinitis, allergic asthma, and pollinosis, which involves the inhibition of the histamine and leukotriene release. Furthermore, another invention was filed to relate cosmetic and dermatological composition of carboxymethyl (CM) naringenin chalcone for the treatment of senile pruritus, psoriasis, chronic urticaria, and atopic dermatitis in topical form. This invention (FR3065372A1) reported several advantages of the application of naringenin chalcone (**1**), including its ability to inhibit cytokine thymic stromal lymphopoietin (TSLP), in turn relieving the itchy sensation associated with atopic dermatitis and subsequently reducing the scratching behavior of patients that causes deterioration of the skin barrier. The importance of this chalcone then led to another patent filed relating to its stability. This invention (JP2003221356A) provides the stabilizing method of **1**, which involves the use of γ-cyclodextrin. The addition of cyclodextrin to the naringenin chalcone (**1**) aqueous solution with constant stirring increases their physical contact and in turn stabilizing the chalcone.

9.9.3 Licorice

There are a number of patents filed on the extraction method of licochalcones. One of the patents is focused on the extraction of licochalonce A (**10**) by thermal processing. The invention (KR100645994B1) described the method to obtain baked licorice by heating the plant at a temperature ranging from 100 °C to 250 °C for 80 to 120 min The licorice extraction method by using a mixture of hexane and ethanol as a solvent with ultrasound instrument was also reported. The yield of the licochalcone A (**10**) can be increased by adjusting the processing time and temperature of the extraction with the solvent allowed in food processing. Another purification method of the same chalcone was filed in another patent (CN105859538B). The method involves the reflux method of crushed *Glycyrrhiza* spp. with solvent, together with purification method of licochalcone A (**10**) and recrystallization method. This method was claimed to be simple and results in shorter production time and higher yield.

Licochalcone A (**10**) is a pharmacologically active compound that gains great interest of researchers. A patent was filed on its application to prepare anticancer

medicines or healthcare products. The invention (CN103768042A) revealed that this chalcone has inhibitory effects on cell proliferation of various tumors such as breast, lung, stomach, and pancreatic cancer and melanoma. Thus, licochalcone A (**10**) is applicable as an anticancer drug or healthcare product preparations for clinical treatment or auxiliary treatment of correlated tumors. Furthermore, an invention (DE10356187A1) recorded that licochalcone A (**10**) could be used as part of the active ingredient as cosmeceutical or dermatological products in combination of phytosterols and aqueous extract from *Glycyrrhizae inflatae*. The invention relates to cosmeceutical or dermatological preparations comprising of active ingredients, particularly toward sensitive and aging skin. According to the invention, it is advantageous to have licochalcone in the form of an aqueous extract with the mixture of licochalonce A (**10**), water, and one or more polyoyls. Another patent was also filed for the active substance with incorporation of licochalcone A (**10**) and phenoxyethanol, relating to a cosmetic and pharmaceutical preparation. This invention (US8969418B2) reported an active substance comprised of licochalcone A (**10**), phenoxyphenol, and glycerine with 0.01–20.00% by weight of active ingredients, and the preparations showed better activity against dyschromia, inflammatory skin conditions, reddening, skin aging, skin dryness, and sensitive skin.

9.9.4 Fingerroot

Some patents related to fingerroot and its chalcones were found. One of the patents was filed on the use of extract of *Boesenbergia pandurata* and its chalcones, panduratin derivatives. The patent (US8653143B2) relates the composition of panduratin derivatives or the extract of *Boesenbergia pandurata* as the active ingredient for the prevention and treatment of a metabolic disease, including obesity, diabetes, hyperlipidemia, and hypercholesterolemia. It also focused on the safety application as the active ingredients in the mentioned diseases besides evaluating their effects on weight and body fat loss, lipid accumulation inhibition and anti-diabetes.

Panduratin A (**19**), a chalcone in fingerroot, is a biological active compound with a few patents filed for its pharmacological usage. An invention (KR100973221B1) was found to relate the novel use of chalcone as antimicrobial and anti-acne agent. The chalcone was claimed to show excellent inhibitory activities against the growth of skin microorganisms, including *Staphylococcus* spp., and can be used to treat acne effectively. Moreover, panduratin A (**19**) was filed for an invention on its anti-atopic dermatitis properties by suppressing pruritus. Atopic dermatitis was characterized by dry skin, erythema, keratinization, and epidermal thickness and wounds. In addition, the same chalcone was reported for its novel use in enhancing muscle growth and exercise performance capability, as well as fighting fatigue according to an invention (EP2572710B1). This patent has assured the safety of the use of panduratin A (**19**), which was isolated from the rhizome of dry *Boesenbergia pandurata* by using distilled water, ethanol, and subcritical water or supercritical carbon dioxide from the oil obtained by pressing the plant. This natural chalcone was proven to enhance

the expression of intramuscular PPAR-δ protein, thus promoting muscle enhancement besides recovering from fatigue and improving the ability of exercise.

9.10 Perspectives

Chalcones are a common class of compounds found to be present in edible plants including tomatoes, apples, licorice, and fingerroot. Its molecular structure of two aromatic rings connected by three carbons with conjugated carbonyl group and double bond gives way for the yellow or orange color of these compounds. The compound is biosynthetically available through chalcone synthase pathway and structurally closely related to flavanone and anthraquinone. A wide range of biological activities are associated with the natural chalcones from food, attributing to the keto-ethylenic group in the structure. The biological activities reported include antioxidant, antimicrobial, anticancer, anti-inflammatory, antihypertensive, neuroprotective, and hepatoprotective effects. Moreover, these chalcones have excellent profile in terms of food safety by showing nontoxicity against normal cells through a number of animal and human clinical studies. The overall beneficial effects of chalcones revealed that these compounds are highly nutritious and safe to be consumed, as well as highly potential lead compounds to be further developed into alternative drugs to treat various diseases.

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Dietary Xanthones

10

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Abstract

Xanthones, polyphenolic compounds, are one of the most important classes in natural products, as they exhibit variety of pharmacological and health benefits, i.e., anti-obesity, antidiabetic, antioxidant, anti-Alzheimer, anti-inflammatory, and anticancer. Xanthones can be found in higher plants that are used as food sources, for example, *Garcinia mangostana* L. (the queen of fruits, mangosteen), a tropical fruit with reddish-purple pericarp that *ca*. 80% of it consists of xanthones. α-, β-, and γ-Mangostin are the major xanthones present

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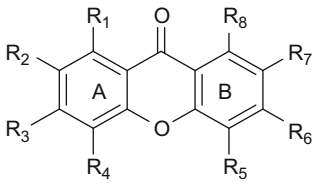
in mangosteen that exhibit potential pharmacological activities. Mangosteen is used as popular food in beverages and in other health products. Also, it is traditionally used as a remedy in treating wounds, inflammation, and bacterial infections. Here in in this chapter, we draw the attention to xanthone compounds present in mangosteen showing their bioactivity and benefits on health, biosynthesis, drug discovery, registered patents, and physicochemical property space.

Keywords

Natural xanthones · Health benefit · Marketed products · Drug discovery · Chemical space

10.1 Introduction

Xanthones are natural polyphenols with the basic molecular formula $C_{13}H_8O_2$ that are commonly found in lichens, fungi, and higher plants (Vieira and Kijjoa 2005). Xanthones have been reported from three main families and seven major genera of higher plants: Anacardiaceae (*Mangifera*), Gentianaceae (*Gentiana* and *Swertia*), and Guttiferae (*Calophyllum*, *Garcinia*, *Platonia*, and *Hypericum*). Since their initial discovery, they have been found in several natural sources aside from *planta* (El-Seedi et al. 2009). For example, new xanthones have been isolated from *Aspergillus versicolor*, the deep-sea-derived fungus (Wang et al. 2016a); from *Talaromyces islandicus*, an endophytic fungus obtained from freshly collected marine red alga *Laurencia okamurai* (Li et al. 2016); and from *Kielmeyera variabilis* from the Clusiaceae family (Coqueiro et al. 2016). Xanthones have basic tricyclic skeleton that encompasses two benzenoid and one pyranoid rings (Fig. 1) (Gales and Damas 2005).



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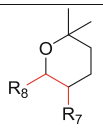
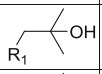
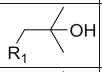
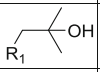
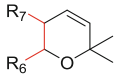
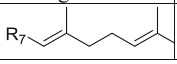
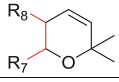
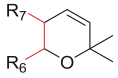
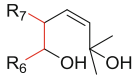
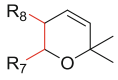
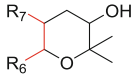
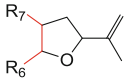
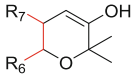
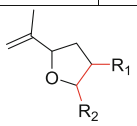
(1)	Prenyl	OCH_3	OH	H	H	OH	Prenyl	OH
(2)	Prenyl	OCH_3	OH	H	H	OCH_3	Prenyl	OH
(3)	Prenyl	OH	OH	H	H	OH	Prenyl	OH
(4)	Prenyl	OCH_3	OH	H	H	OH	 Bicyclic ring	
(5)	OH	H	H	OH	Prenyl	OH	Prenyl	OH
(6)		OCH_3	OH	H	H	OH	Prenyl	OH
(7)		OH	OH	H	H	OH	Prenyl	OH
(8)		OH	OH	Prenyl	H	OH	Prenyl	OH
(9)	Prenyl	OCH_3	OH	H	H	 Bicyclic ring		OH
(10)	OH	H	H	OH	H	OH		OH
(11)	OH	Prenyl	OH	H	Prenyl	OH	 Bicyclic ring	
(12)	Prenyl	OCH_3	H	H	H	OH	Prenyl	OH
(13)	H	OH	H	H	H	OCH_3	Prenyl	OH
(14)	OH	H	H	OH	H	OCH_3	Prenyl	OH
(15)	Prenyl	OH	Prenyl	OH	H	H	H	OH
(16)	Prenyl	OCH_3	OH	H	H	 Bicyclic ring		OH
(17)	Prenyl	OCH_3	OH	H	H	 Bicyclic ring		OH
(18)	Prenyl	OH	OH	H	H	 Bicyclic ring		OH
(22)	Prenyl	OCH_3	OH	H	H	 Bicyclic ring		OH

(23)	Prenyl	OCH_3	OH	H	H	 Bicyclic ring		OH
(24)	Prenyl	OCH_3	OH	H	H	 Bicyclic ring		OH
(25)		OCH_3	OH	H	H	OH	Prenyl	OH
(26)	 Bicyclic ring		OH	H	H	OH	Prenyl	OH
(27)	H	H	H	OH	H	OCH_3	Prenyl	OH
(28)		OCH_3	OH	H	H	OH	Prenyl	OH

10.2 Xanthones Biosynthesis

Xanthone biosynthesis has been examined at the transcriptional, protein, and metabolite levels based on in situ mRNA hybridization, indirect immunofluorescence detection, and mass spectrometry analyses, respectively. The xanthone carbon skeleton is biosynthesized by the action of the enzyme benzophenone synthase (BPS). Both the BPS transcripts and proteins are localized in the innermost endodermal cell layers of the root cortex and the outermost exodermis, respectively. Xanthone metabolites have been reported to be found in the same tissues (Tocci et al. 2018). In *Hypericum perforatum*, the starting substrates for xanthones biosynthesis are acetyl-CoA, isobutyryl-CoA, 4-coumaroyl-CoA, and benzoyl-CoA. The latter is used by BPS, the key enzyme in xanthone biosynthesis (Beerhues 2011); BPS cDNAs have been reported to be cloned from both *Hypericum* and *Garcinia* genera (Nualkaew et al. 2012). *H. androsaemum* BPS was converted to phenylpyrone synthase, a new polyketide synthase (PKS) variant, by a point mutation in the active site cavity (Klundt et al. 2009). The important reactions in xanthone biosynthesis are shown in (Fig. 2) (El-Awaad et al. 2016). The two trihydroxyxanthone products are likely the precursors of all plant xanthones (El-Seedi et al. 2010).

The biosynthesis of neobraclactones A–C, three unmatched xanthones from *Garcinia bracteata*, has been suggested to involve hydration of the double bond

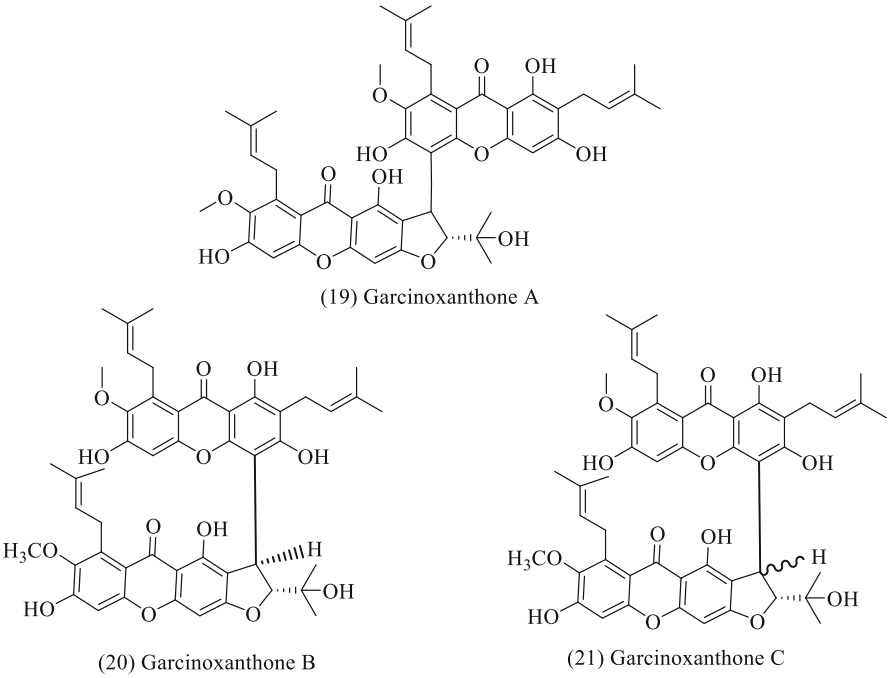


Fig. 1 Xanthone bioactive compounds

between C-8 and C-8a of xanthone 4 to afford intermediate A, followed by a Baeyer–Villiger oxidation-like reaction to generate intermediate B. After that, a nucleophilic attack of the lactone carbonyl of B by 8-OH produce the five-membered γ-lactone unit. After subsequent dehydration and oxidation of the prenyl group, neobractatin (4) is converted into neobraclactones A and B (1–2); then, compound 3 can be generated via cyclization of the 1,1-dimethylallyl group with the ortho-hydroxyl group at C-3, as shown in (Fig. 3) (Niu et al. 2017).

10.3 Biological Activities and Pharmacological Assays of Natural Xanthones

Naturally-occurring xanthones have been reported to have diverse biological activities (Fig. 4). In this section, the biological activities of xanthones are deeply described with an emphasis on their potential utility as therapeutic agents (El-Seedi et al. 2010).

10.4 Mangosteen Containing Xanthones

Garcinia mangostana Linn. commonly called "Queen of fruits and Food of Gods". Mangosteen is a well-known tropical Asian delicious fruit mainly in Indonesia, Malaysia, and Thailand (Fig. 5) (Astuti et al. 2013; Shan et al. 2011).

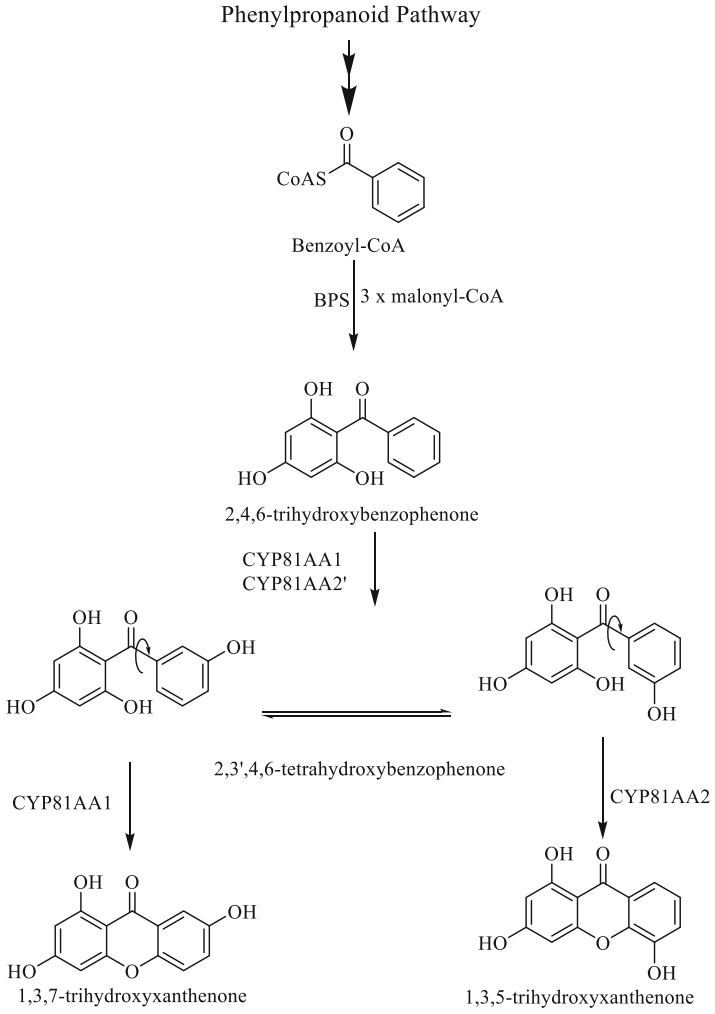


Fig. 2 Xanthone biosynthesis. The crucial enzymes are benzophenone synthase (BPS) that forms the carbon skeleton and cytochrome P450 (CYP81AA(1-2)) that catalyses both 3′-hydroxylation and regiospecific *C–O* phenol coupling

G. mangostana fruits are enriched by xanthones, terpenes, anthocyanins, phenols, tannins, and vitamins (Sarawut Jindarat 2014; Shan et al. 2011). Mangosteen derived xanthones, viz., *α*- and *β*-mangostin predominantly found in roots, barks, edible fruits, and seeds. Mangosteen has been traditionally used for treatment of common cold, diarrhea, cystitis, dysentery, rash, skin infections, and gastrointestinal ailments. Xanthones from mangosteen have been reported as antioxidant, antibacterial especially for *Mycobacterium tuberculosis* and anticancer (Sarawut Jindarat 2014). A previous recent study (Sukma et al. 2011) found new xanthone named γ-mangostin **(3)** in peels of the fruits having anti-inflammatory action besides α **(1)-** and *β* **(2)-**mangostins. The anticancer activity was correlated to xanthones class being natural

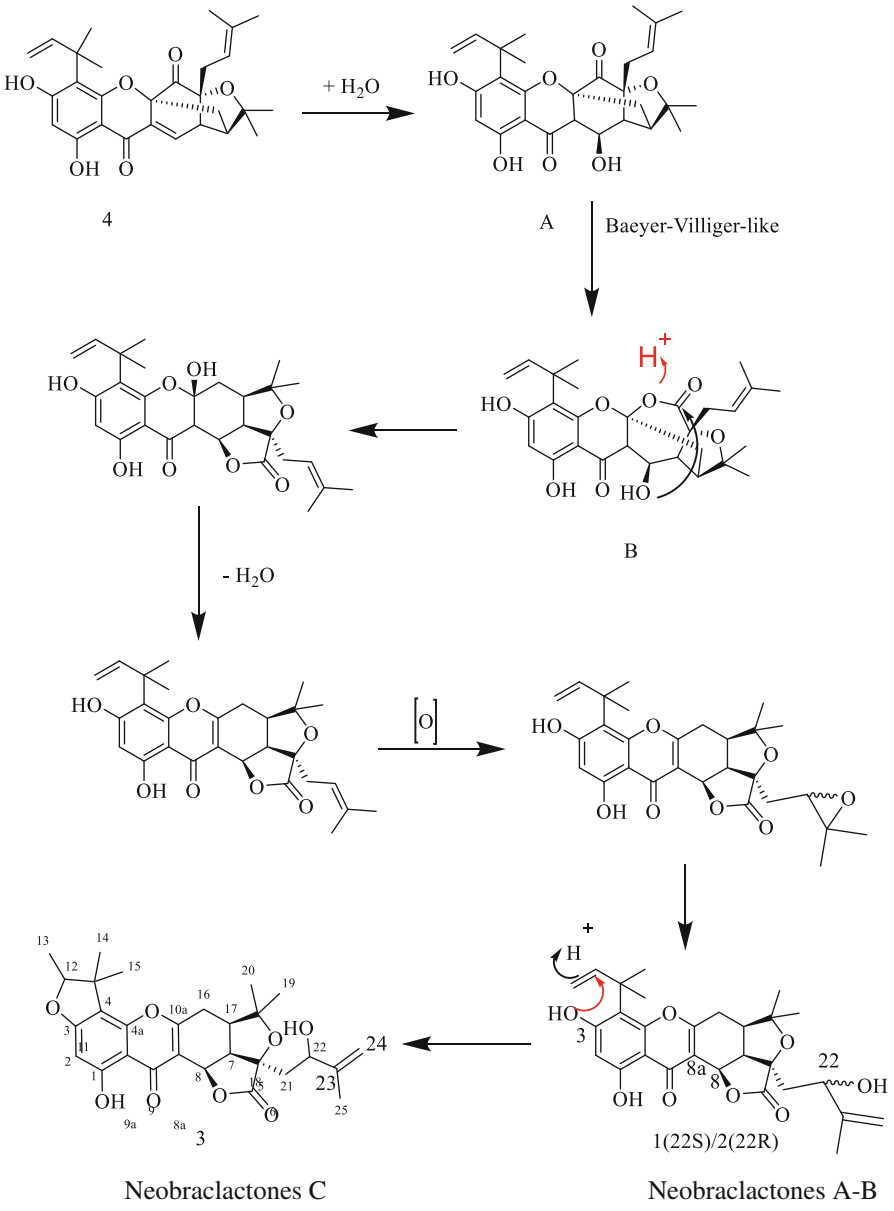


Fig. 3 Plausible biosynthetic pathways of neobraclactones A–C

chemopreventive agent at different stages of carcinogenesis (Shan et al. 2011). Mangosteen nowadays becomes widely known dietary supplement for its effective health benefits. Different mangosteen food supplements are available in the markets in form of juice or capsules under the trade names of XanGo® (XanGo LLC, Orem, UT) or Mangoxan (Pure Fruit Technologies, American Fork, UT).

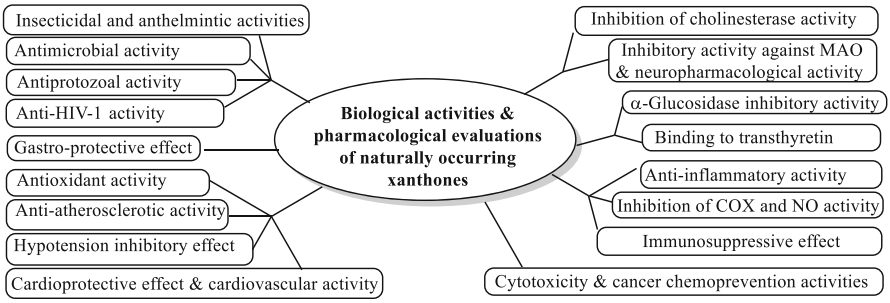


Fig. 4 Biological activities and pharmacological evaluations of naturally occurring xanthones

Fig. 5 *Garcinia mangostana* fruit

10.5 Health Benefits of Mangosteen Fruits Xanthones

10.5.1 Anti-obesity Activity

Obesity is widely recognized as a major public burden caused by an imbalance between energy intake and energy consumption. Obesity leads to severe pathological diseases such as hyperlipidaemia and atherosclerosis (Cooke and Bloom 2006). More than 12 xanthones were isolated from *G. mangostana* L. and evaluated for their inhibitory activities against porcine pancreatic lipase (Chae et al. 2016). These xanthones were shown to suppress the lipid absorption in the small intestine via delaying the hydrolysis of dietary lipid-derived triacylglycerol into glycerol and fatty acids. Furthermore, α-mangostin **(1)** from *G. mangostana* is an effective noncompetitive inhibitor of pancreatic lipase with an IC_{50} value of 5 μM. Additionally, β-mangostin **(2)**, γ-mangostin **(3)**, isomangostin **(4)**, gartanin **(5)**, garcinone D **(6)**, 9-hydroxycalabaxanthone **(9)**, smeathxanthone A **(10)**, tovophyllin A **(11)**, mangostanin **(12)**, and 1,7-dihydroxy-3-methoxy-2-(isoprenyl) xanthen-9-one **(13)** showed moderate activity relative to orlistat as a positive control as in (Fig. 6).

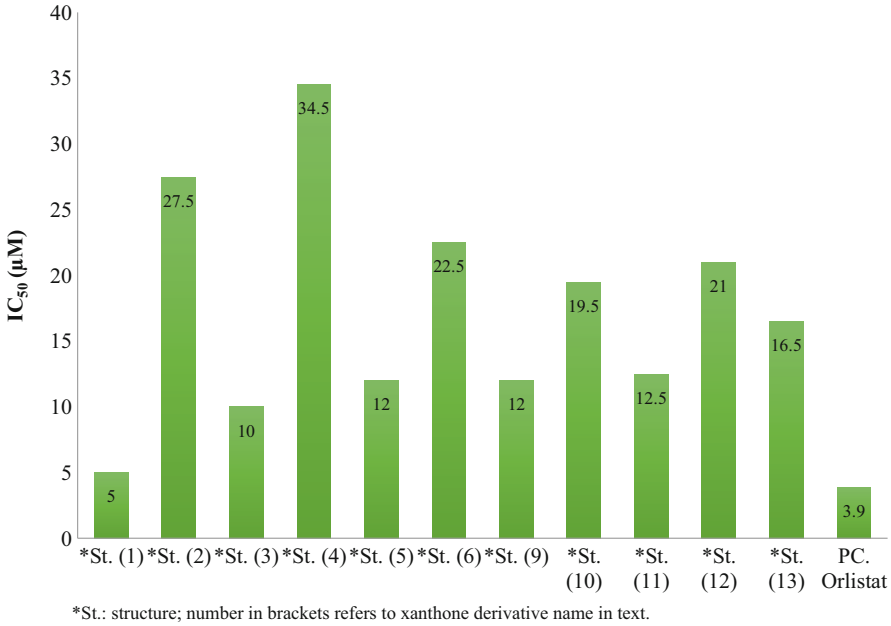
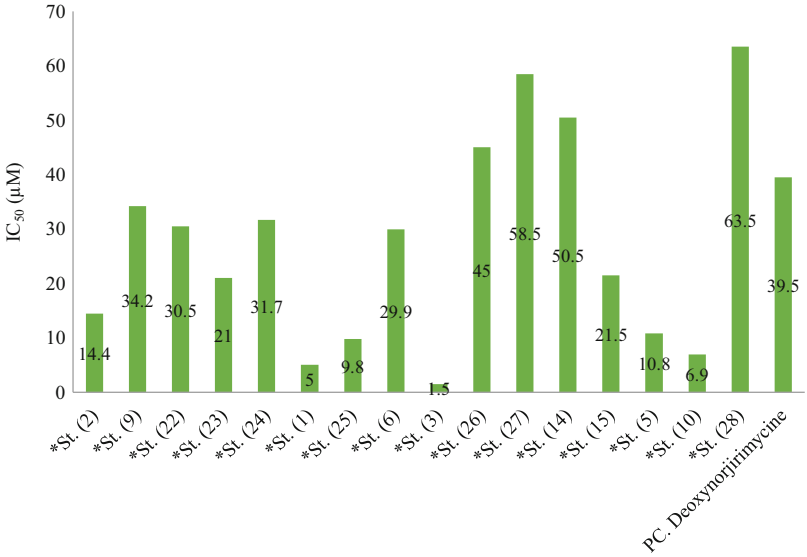


Fig. 6 Pancreatic lipase inhibition assay of *G. mangostana* isolated xanthones L.in comparison to positive control orlistat. (∗St.: structure; number in brackets refers to xanthone derivative name in text)

10.5.2 Antidiabetic Activity

Xanthones are the most abundant class of secondary metabolites in *G. mangostana* L., and the ethanol extract of *G. mangostana* L. showed potent α-glucosidase inhibitory activity with $IC_{50} = 3.2$ µg/ml. α-Glucosidase, a glucosidase found in the small intestine, breaks down starch and disaccharides into glucose (Santos et al. 2018). α-Glucosidase inhibitors are used for the treatment of type 2 diabetes by decreasing the absorption of carbohydrates in the intestine, resulting in a slower and lower increase in blood glucose (Kim et al. 2005). α-Glucosidase inhibition by sixteen xanthones isolated from *G. mangostana* was evaluated. In vitro and in vivo α-glucosidase inhibitory assays for 16 xanthones revealed that mangosteen is a rich source of potent α-glucosidase inhibitors. The most potent of the tested compounds is γ-mangostin **(3)**, which is a substantial improvement over the traditional glycosidase inhibitor deoxynorijirimycin. α-Mangostin **(1)** and the ethanol extract of mangosteen were shown to be effective anti-hyperglycaemic agents in several studies (Ryu et al. 2011). It was observed that better inhibition as free hydroxyl groups exist on A and B xanthone ring, suggestive that the number of free hydroxyl groups influenced the inhibition potency. The alkyl substituent was a more crucial factor for the inhibition. Geranylated xanthone like smeathxanthone A **(10)** is seven times more effective than prenyl xanthone 1,5,8-trihydroxy-3-methoxy-2-(isoprenyl) xanthone **(14)**.



*St.: structure; number in brackets refers to xanthone derivatives name in text.

Fig. 7 α-glucosidase inhibition assay of *G. mangostana* isolated xanthones L.in comparison to positive control deoxnorjirimycin (Ryu et al. 2011). (∗St.: structure; number in brackets refers to xanthone derivatives name in text)

Furthermore, a comparison of prenyl and prenyl hydrate substitution established that prenylation gave more effective inhibitory activity in comparison to the positive drug control, deoxynojirimycin IC_{50} (39.5 ± 0.5 μM), as shown in (Fig. 7).

10.5.3 Antioxidant and Anti-Alzheimer Activities

Redox homeostasis is maintained in all biological systems as an intracellular equilibrium. Excessive oxidative stress can lead to severe damage in DNA and alteration of endogenous macromolecules such as proteins and lipids. Xanthones have been described as effective antioxidants, as they are capable of decreasing the cellular ROS levels. The antioxidant activity of xanthones can be useful for treatment of cardiovascular and neurodegenerative diseases, inflammation, atherosclerosis, and cancer. Phyu and Tangpong studied the effect of xanthones from *G. mangostana* and its antioxidant activity which allowed protection of the brain and nervous system from lead poisoning (Phyu and Tangpong 2014). They concluded that xanthones from *G. mangostana* showed potent effect on Pb-induced acetylcholinesterase (AChE) dysfunction and memory loss, and oxidative stress inhibition. It was found that xanthones are comparable to vitamin E in decreasing the symptoms of lead poisoning and because xanthone is water soluble rather than vitamin E posing xanthones as of better tolerance and bioavailability. α-Mangostin **(1)**,

8-deoxygartanin **(15)**, gartanin **(5)**, garciniafuran **(16)**, garcinone C **(7)**, garcinone D **(6)**, and γ-mangostin **(3)** were isolated by Wang and coworkers (Wang et al. 2016b), showed potent inhibition of self-induced β-amyloid (Ab) aggregation and also β-site amyloid precursor protein-cleaving enzyme 1, and acted as potential antioxidants and biometal chelators. α-Mangostin, γ-mangostin, gartanin, and garcinone C showed stronger antioxidant activity to DPPH free radical than the standard Trolox and ROS species, and strong neuroprotective effects against glutamate-induced HT22 cell death, also gartanin, garcinone C, and γ-mangostin were able to penetrate the blood–brain barrier (BBB). Due to these various pharmacological activities of xanthones and their lyophilic nature, they could have used as promising agents for treating Alzheimer's disease (Wang et al. 2016b) (Table 1).

10.5.4 Anticancer Activity

Cancer in general is a term applied of malignant diseases involving irregular cell growth causing tumor formation with the possibility to invade or extend to other parts of the body (Aboul-Enein et al. 2012). According to WHO, cancer is one of the deadliest diseases with a high mortality rate globally; 1.69 million deaths from lung cancer, 788,000 deaths from liver cancer, 774,000 deaths from colorectal cancer, 754,000 deaths from stomach cancer, and 571,000 deaths from breast cancer occur each year, making the development and discovery of new, patentable anticancer drugs with higher efficacies a pressing need (Roleira et al. 2018). As a green method and since the discovery of penicillin from *Penicillium notatum* fungus, compounds purified from natural sources have provided a wealth of bioactive molecules (Davison and Bewley 2018); plants, as a source of natural compounds, have provided medicinal science with many lead compounds and drug molecules, especially anticancer agents (Khazir et al. 2014) i.e., epipodophyllotoxin, vinca alkaloids (vinblastine and vincristine) from *Catharanthus roseus* and camptothecin quinolone, as well as the famous anticancer drug Taxol (*Taxus brevifolia*) (Sharma et al. 2017). Due to the increasing interest in functional foods to treat ailments and improve health, scientists have directed their attention toward *G. mangostana* as a source of polyphenolic compounds such as xanthones that can be used to prevent and treat cancer (Mohamed et al. 2017; Rasheed et al. 2018). Mangostanaxanthone IV **(17)**, *β*-mangostin **(2)**, garcinone E **(8)**, and α-mangostin **(1)** from *G. mangostana* exhibited significant antiproliferative and cytotoxic activities via the induction of apoptosis and necrosis in both HepG2 (human hepatocellular carcinoma), HCT116 (colorectal adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cells, as shown in (Table 2) (Mohamed et al. 2017).

In a previous study, among 14 tested compounds from *G. mangostana*; 7-*O*-demethyl mangostanin **(18)** showed significant cytotoxic activity against seven cancer cell lines, CNE-1, CNE-2 (human malignant glioma cell), A549, H460 (lung cancer cell line), PC-3 (prostate cancer cell line), SGC-7901 (gastric cancer cell line), and U87 (human malignant glioma cell), and it was better than the positive control hirsutanol A as shown in (Fig. 8) (Yang et al. 2017).

Table 1 Antioxidant and anti-Alzheimer assays of xanthones from *G. mangostana* L.

Bioactive xanthones	Biological activity				
	Inhibitory against Aβ42 self-induced aggregation in tube	Inhibitory against Aβ42 in *E. coli* cells	BACE1 inhibition rate (%)	Antioxidant	
α-Mangostin **(1)**	= 16.8% PC. Congo red= 86.5%	= 30.2 ± 8.2%	= 60.3 PC. Minocycline= 45.9%	Not active	Not active
8-Deoxygartanin **(15)**	= 4.4% PC. CR.= 86.5%	NA	=16.9 PS. Minocycline= 45.9%	Not active	Not active
γ-Mangostin **(3)**	= 47.8% PC. CR.= 86.5%	= 50.8 ± 7.6%	= 42.1 PC. Minocycline= 45.9%	DPPH radicals scavenging capacity (%) = 23.7 ± 2.7 Standard Trolox= 22.8 ± 0.9	BBB permeabilities Pe (10^{-6} cm s^{-1}) in vitro= 11.95 ± 0.86(CNS +)
Garcinone D **(6)**	= 34.9% PC. CR.= 86.5%	NA	= 62.7 PC. Minocycline= 45.9%	Not active	Not active
Garcinone C **(7)**	=83.7% PC. CR.= 86.5%	=55.9 ± 5.8%	= 18.7 PC. Minocycline= 45.9%	DPPH radicals scavenging capacity (%) = 48.7 ± 0. 5 Standard Trolox= 22.8 ± 0.9	Pe (10^{-6} cm s^{-1}) = 12.63 ± 0.80 (CNS+)
Garciniafuran **(16)**	= 50.7% PC. CR.= 86.5%	NA	= 36.3 PC. Minocycline= 45.9%	Not active	Not active
Gartanin **(5)**	= 63.5% PC. CR.= 86.5%	= 25.4 ± 6.7%	= 39.8 PC. Minocycline= 45.9%	DPPH radicals scavenging capacity (%) = 50.7 ± 1. 4 Standard Trolox= 22.8 ± 0.9	Pe (10^{-6} cm s^{-1}) = 5.91 ± 0.68 (CNS+)

Table 2 Cytotoxic potential using SRB-U assay of xanthones from *G. mangostana* L.

Bioactive xanthones	Cytotoxic activity evaluated by SRB-U assay		
	MCF-7 cell line (μM)	HCT-116 (μM)	HepG2 cell line (μM)
Mangostanaxanthone IV **(17)**	IC_{50}= 90.8 ± 3.9	IC_{50}= 45.7 ± 3.5	IC_{50}= 116.4 ± 6.7
β-Mangostin **(2)**	IC_{50}= 97.2 ± 2.9	IC_{50}= 72.6 ± 6.2	IC_{50}= 72.6 ± 6.2
Garcinone E **(8)**	IC_{50}= 15.9 ± 3.8	IC_{50}= 15.8 ± 1.0	IC_{50}= 16.7 ± 0.9
α-Mangostin **(1)**	IC_{50}= 51.5 ± 2.8	IC_{50}=68.9 ± 2.9	IC_{50}= 53.8 ± 3.2

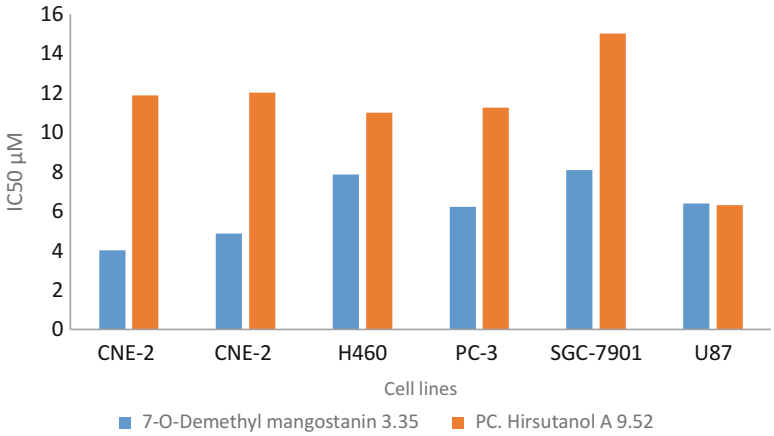


Fig. 8 Cytotoxic effect of 7-*O*-demethyl mangostanin (18) against positive control hirsutanol A using MTT assay (Yang et al. 2017)

As reported, xanthones isolated and purified from *G. mangostana* exhibited variety of biological activities using different bioassays (Chin and Kinghorn 2008). Antioxidant activity using the authentic and morpholinosyndnonimine (SIN-1)-derived peroxynitrite assays on 14 isolated xanthones from *G. mangostana* were evaluated, followed by evaluation of α-mangostin **(1)** specially for its worthy potency on SIN-1 derived peroxynitrite test (IC_{50} <0.49 μM), using mouse mammary organ culture (MMOC) assay, and was found to exhibit inhibition of 7,12 dimethylbenz[α] anthracene-induced preneoplastic alveolar lesions in the MMOC assay, exhibiting an IC_{50} = 2.4 μM of 1.0 μg/ml (Jung et al. 2006; Kinghorn et al. 2011). Also, γ-mangostin **(3)** was found to exhibit potent antiproliferative activity lower than the clinical chemotherapeutic agent carmustine, against human malignant glioblastomas (MGs), using U87 MG and GBM 8401 assays. The IC_{50} values of γ-mangostin were 74.14 ± 2.93 and 64.67 ± 2.42 μM to that of the control drug, carmustine (BCNU), whose IC_{50} values were 632.1 and 346.6 μM in U87 MG and GBM 8401 cells (Chang et al. 2010). The major xanthones in edible mangosteen fruit, α- and γ-mangostin,

Table 3 Dimeric xanthones (garcinoxanthones A–C) showed potent inhibition of LPS-induced NO production in RAW246.7 cells compared to indomethacin

Naturally occurring xanthone	Inhibitory effect on LPS-induced NO production in RAW264.7 cell line: IC_{50} (μM)
Garcinoxanthone A **(19)**	$IC_{50} > 20$
Garcinoxanthone B **(20)**	11.3 ± 1.7
Garcinoxanthone C **(21)**	18.0 ± 1.8
Indomethacin	3.9 ± 0.3

exhibited valuable dietary source as they have multi-activity on different bioassays relevant to cancer chemoprevention (Mohamed et al. 2017), antimalarial (Riscoe et al. 2005), and so on. So further in vivo bioassays investigations for xanthones in *G. mangostana* is required to reach applications in drug discovery (Kinghorn et al. 2011).

10.5.5 Anti-inflammatory Activity

Inflammation is a protective response of the body against infection, and it is usually associated with effects such as swelling, pain, increased vascular permeability, protein denaturation, and membrane alteration (Chahar et al. 2012). Pro-inflammatory cytokines, i.e., IL-1β, IL-6, and TNF-α, and inflammatory mediators, including ROS, NO, and PGE2, from nitric oxide synthase (NOS) and COX-2 pathways are produced by pro-inflammatory cells called macrophages; NO is used as a defence response by many pathogens, but its overproduction may cause chronic inflammation (Ee et al. 2011). Xanthones show a variety of activities against NO production.

Liu and co-workers isolated three dimeric xanthones, garcinoxanthones A–C **(19-21)**, and four monomeric xanthones, garcinoxanthones D–G, from *G. mangostana* pericarps (Table 3) (Liu et al. 2016). These rare dimeric xanthones showed potent inhibition of LPS-induced NO production in RAW246.7 cells compared to indomethacin. The significant activities of the dimeric compounds have led to such new classes of xanthones being studied both in vitro and in vivo for utilization in healthy foods and pharmaceutical applications.

10.6 Xanthone Drug Discovery and Improvement

Highly oxygenated xanthones can additionally play an important role in drug discovery and natural products chemistry serving as a key intermediate for the synthesis or derivatization to produce other important classes of drugs (Sahin et al. 2009). Studies on the reactivity of xanthones and their application as key intermediates in the synthesis of highly functionalized structures have mostly focused on the reactivity of its carbonyl group (Kang and Fang 1997; Sousa and Pinto 2005) and to lesser extent, the ether function. Moreover, xanthone nucleus was found to be

involved in the synthesis of fused naphthalenes as well as isoquinolines (Sousa and Pinto 2005). Examples on xanthone utilization as starting material for the synthesis of complex other natural products include mycotoxins, i.e., diversonol, blennolide C4, and hemisecalonic acids (Ohnemueller et al. 2007). With regards to xanthones derivatization to improve its pharmacokinetic properties and its bioavailability considering its relatively low polarity, several approaches have also been reported mostly targeting the reduction of its particle size, nano-formulation, or addition of surfactants. Most of the reports on improvement of xanthone pharmacokinetics have focused on either α-mangostin **(1)** or mangiferin. Functionalized gold nanoparticles of magniferin (a xanthone derivative isolated from *Mangifera indica* leaves) were attempted as drug delivery method and found non-toxic to normal breast cell line (MCF-10A) (Patra et al. 2018). Self-assembled phospholipidic nano-mixed miceller system (SPNMS) of mangiferin functionalized with co-delivery of vitamin E was also found to improve the bioavailability and ultimate biopharmaceutical performance of magniferin in rats for an enhancement of its anticancer effect (Khurana et al. 2018). Similarly, *in vivo* application of α-mangostin is limited due to its hydrophobic nature, poor aqueous solubility, and stability and thus low bioavailability affecting its accumulation in the target organs. Nanoencapsulation of α-mangostin into the core of poly(ethylene glycol)–poly(l-lactide) (PEG–PLA) nanoparticles improved its bioavailability in both the brain and liver, reduced Aβ deposition, attenuated neuroinflammatory responses, ameliorated neurologic changes and suggesting that nanotechnology can improve xanthones therapeutic efficacy, particularly in management of Alzheimer's disease (Yao et al. 2016). Other drug applications of nanoparticles encapsulation of α-mangostin include its application as an oral drug delivery system for the chemoprevention or treatment of colon and prostate cancers (Qiu et al. 2016). α-Mangostin self-microemulsion was evaluated as a drug loading system to improve its pharmacokinetic performance and to enhance its serum bioavailability and moreover its distribution in lymphatic organs (Xu et al. 2017). In terms of sustaining α-mangostin local release for acne treatment, nanoparticles of α-mangostin prepared using ethyl cellulose-methyl cellulose polymer were also found successful to sustain its release into synthetic sebum (Pan-In et al. 2015). A soft capsule, with vegetable oil as the dispersion matrix, was prepared to improve the bioavailability of α-mangostin and was found effective to improve its bioavailability and its tissue distribution i.e., small intestine, ovaries, stomach, and bone (Zhao et al. 2016). Inclusion of other xanthones in such nano-formulations has yet to be applied to improve its efficacy and or biodistribution.

10.7 Mangosteen Registered Patents

Multiple patents have been earned by different pharmaceutical companies regarding preparation, extraction and pharmacological activities correlated to xanthones particular mangostins constituents. Inventors of Mangoxan® registered a patency for nutraceutical composition of Mangosteen fruit pulps and pericarp combination. The inventors correlated the main health benefits of mangosteen fruits due to the presence

of main active ingredient xanthones (Garrity et al. 2004). Another patency was registered for preparation of nutraceuticals enriched with mangostins from *G. mangostana* due to its potent antioxidant and anti-inflammatory actions (Gokaraju et al. 2014). A patent was registered for novel extraction procedures of mangostin xanthones rich extracts (Moffett and Shah 2006). Another one for novel production of γ-mangostin xanthones from *G. mangostana* fruits have been registered since previously proven antioxidant activity (Gokaraju et al. 2013).

10.8 Physiochemical Property Space

Recent trends have shown a potential in analyzing a group of compounds based on their physicochemical properties. The tricyclic skeleton is a central core structure in xanthones and unifies these compounds chemically. Hence, an introduction of more properties should provide more useful analytical tools that help in exploring this particular group of compounds. The added value is not only in providing a new aspect to investigate a possible correlation but also to predict a possible pharmacological action that could provide a new feature in such compounds. Many correlations can be established for a certain group of compounds taking the advantage of several properties that can be included. Xanthones are no exception. In this section we used web-based principle components analysis, namely, Chemical Global Positioning System of Natural Products (ChemGPS-NP) (Larsson et al. 2007). The ChemGPS-NP provides a wide range of molecular descriptors describing physical–chemical properties of the investigated compounds. Molecular and physicochemical properties incorporated chemically intuitive descriptors, described in detail by Larsson and co-authors. These descriptors include molecular weight; sum of atomic van der Waals volumes (scaled on C atom); sum of atomic Sanderson electronegativities (scaled on C atom); sum of atomic polarizabilites (scaled on C atom); mean atomic van der Waals volume (scaled on C atom); mean atomic Sanderson electronegativity (scaled on C atom); number of atoms; number of non-hydrogen atoms; number of bonds; number of non-hydrogen bonds; number of multiple bonds; aromatic ratio; number of rings; number of rotatable bonds; notable bond fraction; number of double bonds; number of aromatic bonds; number of carbon atoms; number of nitrogen atoms; number of oxygen; number of halogens; number of benzene-like rings; number of aromatic carbon atoms (sp2) n amid; number of amides; number of aliphatic hydroxyls; number of aromatic hydroxyls; number of donor atoms for hydrogen bonds (N and O); number of acceptor atoms for hydrogen bonds (N, O, and F); unsaturation index; hydrophilic factor; Ghose–Crippen molar refractivity; topological polar surface area using N and O; topological polar surface area using N, O, S, and P; Ghose–Crippen octanol/water partition coefficient; and Lipinski Alert Index (drug-like index). The selected ChemGPS-NP descriptors were calculated for all sets of compounds using the software dragonX by Talete srl. (http://www.talete.mi.it). Compounds were submitted on the basis of their structure information as simplified molecular input line entry specification (SMILES; http://www.daylight.com/smiles/) and initially verified as described by Rosén and coworkers

(Rosén et al. 2009) as to omit salts, hydration, and stereochemical and ionic information. Principal component and PCA score prediction were calculated employing the software SIMCA-P+, with the training set ChemGPS-NP. Prior to PCA determination, all data were centered and scaled to unit variance. Navigation through the chemical property space formed by the first three principle components shows obvious deviation with garcinoxanthone C as they distance themselves from the group of xanthones in this chapter (Fig. 9).

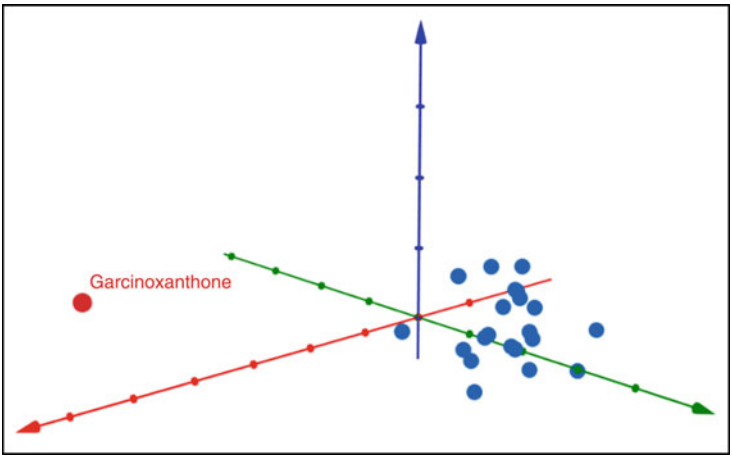


Fig. 9 The remarkable deviation of garcinoxanthone from the rest of the xanthones in the chemical property space where PC1(x=red), PC2(y=green) and PC3(z=blue)

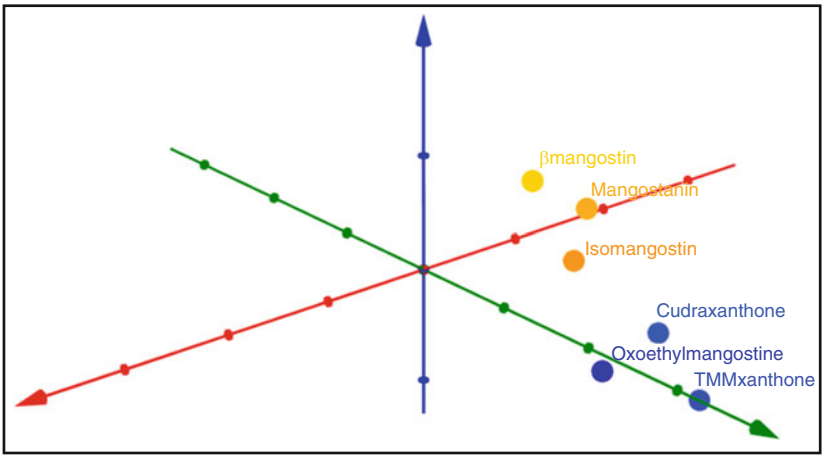


Fig. 10 Anti-obesity and antidiabetics, activity spaces within the chemical property space of xanthones. The fading shade grade shows decrease in activities while the intense shade shows the higher

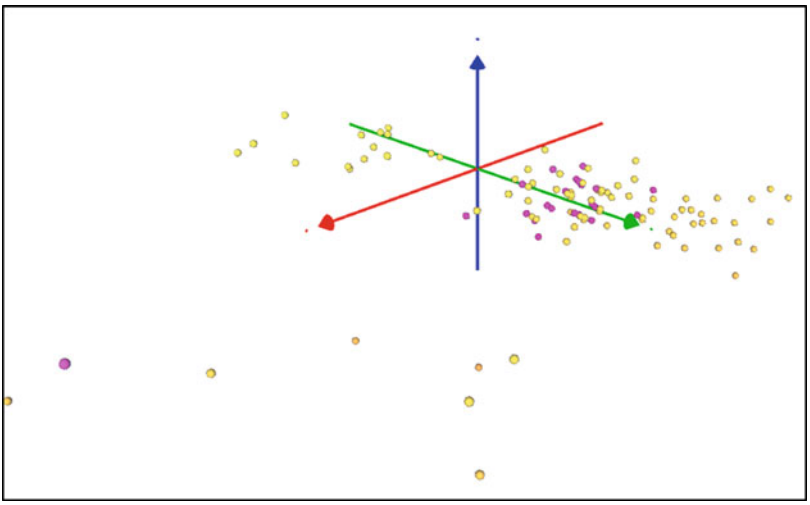


Fig. 11 Activity space determined with bioactive xanthones (anti-obesity and antidiabetics) in pink as compared with the yellow spheres of xanthones in general

The target correlation was established between anti-obesity and antidiabetics, as presented by plotting highest activities of the main compounds. Two distinct spaces were identified referring to unique activity spaces (Fig. 10). Such correlation can be advantageous to predict similar activities for compounds that close or in subspace.

In the Fig. 11 we are able to determine the "activity space" by plotting the active compounds and defining that enclosed space. The activity space can further predict type and extent of activity of any xanthone compounds by simple comparison by two simple questions, firstly, "Does the compound belong to the activity space or not?" and secondly "How far is the compound location from the central of activity space?" – i.e. the further is the distance, the lower could be the activity. The activity space studied here belongs to the anti-obesity and antidiabetics.

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Prenylated Flavonoids in Food

11

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Abstract

Prenylflavonoids, which are also often referred to as prenylated flavonoids, are one of the classes of naturally occurring flavonoids that are widely distributed in plants. They possess at least one prenyl group on the flavonoid skeleton and have various reported bioactivities. Several prenylated flavonoids have been reported from edible plant species, such as *H. lupulus*, *G. glabra*, *M. alba*, *A. heterophyllus*, *G. max*, etc., and their efficacy for promoting human health has been discussed by mainly investigating their bioactivities. This chapter summarizes the bioactivities, bioavailabilities, and metabolism of the prenylated flavonoids from *H. lupulus*, *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max*, as well as their safety, application in foods, and some restrictions.

Keywords

Food sources · Prenylated flavonoids · Bioactivity · Application · Safety

11.1 Introduction

Prenylated flavonoids are one of the classes of flavonoids produced by plants and are widely distributed in the Leguminosae, Moraceae, Euphorbiaceae, Guttiferae, and Umbelliferae plant families (Sasaki et al. 2011). They are also often called prenylflavonoids and possess at least one prenyl group, constituted by a dimethylallyl (3-methylbut-2-en-1yl) unit with five carbons on the flavonoid skeleton. In general, *C*-prenylation occurs on the flavonoid skeleton, and *O*-prenylation has also been reported (Barron and Ibrahim 1996; Yang et al. 2015). In the prenylated flavonoids, the prenyl group is also often cyclized to form a pyran ring with the flavonoid skeleton.

Flavonoids are not only a biologically important family of compounds for plant defense but also pharmaceutically important natural products with various properties, including antitumoral, antiviral, anti-inflammatory, antibacterial, antifungal, anti-osteoporotic, antithrombogenic, antiatherosclerotic, and other activities (Agrawal 2011). In most cases, the prenylation(s) of the flavonoids improve their biological activity (Mukai 2018). An example is the *C*-prenylation of the methylflavonoids isolated from the roots of *Desmodium caudatum*: The *C*-prenylation of the methylflavonoids enhanced the antifungal activities against *Trichophyton* sp. by fourfold over that of the flavonoids without a prenyl group, due to the enhanced binding affinity to biological membranes and improved interaction with target proteins by the increased lipophilicity (Sasaki et al. 2012).

Several prenylated flavonoids have been reported from edible plant species, namely, *H. lupulus*, *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max*. Their impact on human health has been discussed on the basis of the bioactivities of the phytochemicals found in these plants. Among them, *H. lupulus* (Hop) (Fig. 1A) is a flowering plant belonging to the Cannabaceae family and is native in Europe, Southwestern Asia, and North America. It goes without saying that the female cone fruits of *H. lupulus* have been utilized to produce beers. The addition of hops to beverages, especially beer, is largely to impart the distinctive bitter taste and

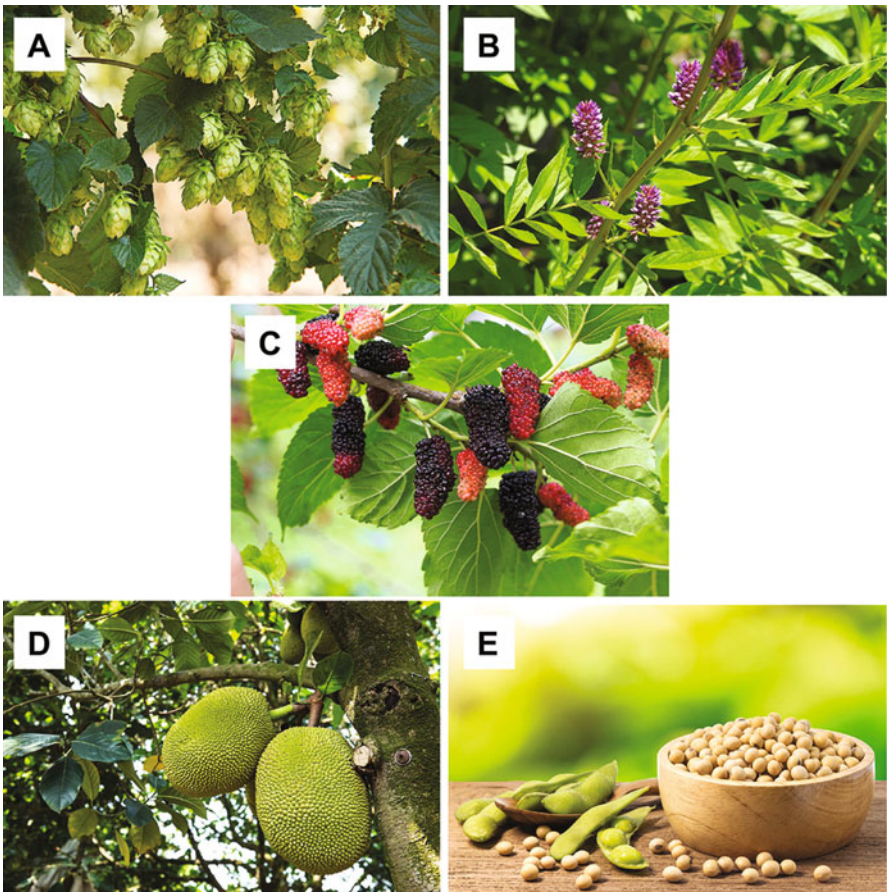

Fig. 1 Photos of edible plant species containing prenylated flavonoids. (**A**) Hop (*H. lupulus*), (**B**) licorice (*G. glabra*), (**C**) white mulberry (*M. alba*), (**D**), jackfruit (*A. heterophyllus*), (**E**) soybean (*G. max*)

aroma, to precipitate certain nitrogenous constituents of the wort, to act as filter aid in the hop back, to confer antibacterial properties to beer, and to assist in the stabilization of the wort (Almaguer et al. 2014). In addition, *H. lupulus* has been traditionally utilized to relieve excitability and restlessness associated with headache, to improve appetite and digestion, and to relieve toothache, earache, and neuralgia (Barnes et al. 2007; Grieve 1971), as well as to remedy sleeplessness and nervousness (Blumenthal and Busse 1998; Zanoli and Zavatti 2008). Further applications of hops for diuretic, antispasmodic, aphrodisiac, sedative, anti-inflammatory, antirheumatic, and anti-pneumonia purposes have also been reported (Zanoli and Zavatti 2008; Bown 2001; Blumenthal and Busse 1998; Weiss 1988; Duke 1985).

G. glabra (licorice) (Fig. 1B) belongs to the family Leguminosae and is native to Southern Europe and parts of Asia. The *G. glabra* root is widely utilized in the food industry as a flavoring agent and confectionary. The first recognized medicinal use of

licorice has been traced back to ancient Assyrian, Egyptian, Chinese, and Indian cultures (Fiore et al. 2005). Licorice is used in traditional medicine for the treatment of some diseases, such as those involving the genital-urinary, gastrointestinal, respiratory, and cardiovascular systems, as well as skin and eye sicknesses (Fiore et al. 2005). Barnes et al. also reported the usage of licorice as an expectorant, demulcent, antispasmodic, anti-inflammatory, and laxative (Barnes et al. 2007). Furthermore, licorice reportedly plays critical roles in the treatments of dyspepsia, and gastric and duodenal ulcers, as well as in the prevention of toxin accumulation in the liver, the reduction of allergic reactions, and the recovery from fatigue and debilitation (Xiaoying et al. 2017). In addition, licorice reportedly possesses efficacy for the treatments of chronic gastritis, peptic ulcer, colic, and primary adrenocortical insufficiency, as well as the accumulation of phlegm associated with coughs and bronchial catarrh (Barnes et al. 2007; Xiaoying et al. 2017).

M. alba (white mulberry) (Fig. 1C) is a moderately fast-growing deciduous shrub or tree growing up to 20–35 m that belongs to the Moraceae family. *M. alba* is commonly found in Korea and China, as well as in warm temperate and subtropical zones of Europe, Asia, and America, and is mainly cultivated in Southeast and East Asian and Middle Eastern countries (Muller 2001). *M. alba* has a wide range of usages and is especially valued for its edible fruits and medicinal properties. Its sweet-tasting fruits are consumed in the raw, fresh, or dried form and are utilized for beverage production, while the foliage is fed to silkworms in the silk-making industry (Ercisli 2004). The fruits of this plant are commonly converted to syrup and used as a honey alternative in Central, Northeast, and Southeast Anatolia (Ercisli 2004). The fruits are also fermented to produce beer, jams, and jellies, while the root extract is used for the treatment of inflammation, diabetes, high blood pressure, and AIDS (Srivastava et al. 2006). Powder from *M. alba* leaves is used in Chinese traditional medicine for treating obese diabetic and hypertensive patients. The leaves are also used in Indian cuisine, such as curry, *saag*, *pakoda*, *paratha*, and *dhokla* (Srivastava et al. 2006; Yen et al. 1996).

A. heterophyllus (jackfruit) (Fig. 1D) also belongs to the same family as white mulberry and is indigenous to South and Southeast Asia. The jackfruit tree reportedly originated in either the rain forests of the Western Ghats in Southwestern India or Malaysia. This plant was progressively introduced to other countries and is currently cultivated in Asia, Australia, the Caribbean region, and America (Azad et al. 2007; Baliga et al. 2011). This plant species has many dietary and medicinal uses. The seeds and young fruits are used as vegetable curries and pickles, while the fresh fruits are consumed in the raw form and in fruit salads. Furthermore, the ripened fruits are converted to syrup, whereas the pulps are consumed as the dried material, or incorporated into juice, biscuits, leather, toffee, and flavors for beverages and ice cream (Azad et al. 2007; Prakash et al. 2009). In India, jackfruits are popular foods and are ranked third in annual production, after banana and mango. In Bangladesh, it is an extremely important tree, and the fruits are widely consumed (Baliga et al. 2011). Most of the parts of the jackfruit tree reportedly possess medicinal properties and have been utilized in the preparation of various old holistic ("whole-body") Indian folk healing systems, Ayurvedic and Yunani medicines

(Saxena et al. 2009). Ripe jackfruits are used to prevent the excessive formation of bile, to develop fresh phlegm, to strengthen the body, and to increase virility. Seed and bark extracts are utilized to aid digestion and for the treatment of diarrhea and dysentery.

G. max (soybean) (Fig. 1E) is the plant that provides soybeans for making soy-based foods (Chen et al. 2012). *G. max* belongs to the Fabaceae family and is native to Southeastern Asia. Its dietary value has been recognized in Asian countries for more than 5,000 years. It is claimed that, in China, the Chou dynasty developed several fermentation methods to make soybeans in different dietary forms, such as tempeh, miso, and tamari soy sauce, before the discovery of tofu in the second century (Badole and Bodhankar 2013). Soybean was introduced to the USA between 1765 and 1800 (Badole and Bodhankar 2013; Brachfeld and Choate 2007) and to Europe in the 1700s (Badole and Bodhankar 2013). One of the important roles of soybean was its implementation in crop rotation as a nitrogen fixator, to restore nitrogen-depleted soil. Several traditional medicine applications of soybean and its constituents include osteoporosis prevention (Wong et al. 2009), bone mineral density restoration in menopausal women (Wong et al. 2009), anti-inflammation (Liao et al. 2009), cancer prevention (Zou and Chang 2011; Sakamoto et al. 2010), and as an antioxidant (Dixit et al. 2012; Takahashi et al. 2005). Other parts of this plant also have been utilized in treatments for various diseases in traditional medicine (Baliga et al. 2011).

This chapter mainly highlights the bioactivities, bioavailabilities, and metabolism of prenylated flavonoids in *H. lupulus*, *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max*, as well as their safety, application in foods, and some restrictions. Their marketed products, as well as patents, will also be discussed in this chapter.

11.2 Bioactive Dietary Prenylated Flavonoids

Several dietary prenylated flavonoids with good pharmacological activities have been isolated from *H. lupulus*, *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max*. Xanthohumol (**1**) and desmethylxanthohumol (**2**) are examples of bioactive *C*-prenylated chalcones with a 3,3-dimethylallyl moiety at C-5′, isolated from *H. lupulus* (Stevens et al. 1997; Stevens and Page 2004; Zanoli and Zavatti 2008) (Fig. 2). Two flavanone types of prenylated flavonoids with a 3,3-dimethylallyl moiety at C-8, isoxanthohumol (**3**) and 8-prenylnaringenin (**4**), were also isolated from this plant (Stevens et al. 1997; Stevens and Page 2004; Zanoli and Zavatti 2008). In addition to the prenylated chalcones and flavanones, phytochemical investigations of *H. lupulus* reportedly indicated the presence of 6-prenylnaringenin (**5**), with a 3,3-dimethylallyl moiety at C-6 of the flavanone backbone structure (Stevens et al. 1997; Stevens and Page 2004; Zanoli and Zavatti 2008).

A phytochemical investigation of *G. glabra* revealed that this plant contains licoflavone A (**6**), which is the flavone type of **5** (Xiaoying et al. 2017) (Fig. 3). Furthermore, licochalcone A (**7**) with a 1,1-dimethylally moiety at C-5′ and

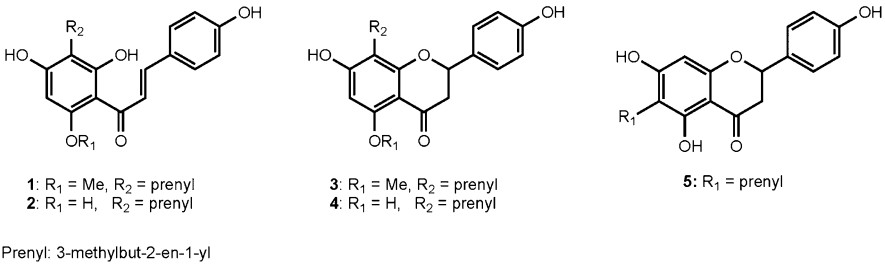

Fig. 2 Examples of bioactive dietary prenylated flavonoids (**1**–**5**) of *H. lupulus*

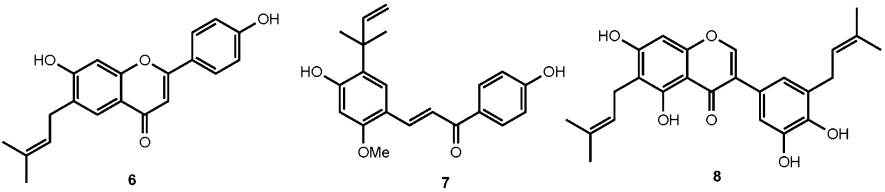

Fig. 3 Examples of bioactive dietary prenylated flavonoids (**6**–**8**) of *G. glabra*

isoangustone A (**8**) with two 3,3-dimethylallyl moieties at C-6 and C-5′ of the isoflavone skeleton have also been isolated from *G. glabra* (Xiaoying et al. 2017).

Flavone types of prenylated flavonoids, such as 3′-geranyl-3-prenyl-2′,4′,5,7-tetrahydroxyflavone (**9**), 3′,8-diprenyl-4′,5,7-trihydroxyflavone (**10**), cyclomulberrin (**11**), sanggenons J (**12**) and K (**13**), cyclomorusin (**14**), morusin (**15**), atalantoflavone (**16**), and kuwanon G (**17**), have been isolated from *M. alba* (Butt et al. 2008; Dat et al. 2010) (Fig. 4). The prenyl moiety of most of the prenylated flavones is cyclized to form the pyran ring, as observed in the structures of **11**–**16**, and this is one of the characteristic structural features of the isolates from *M. alba*. The *C*-geranylated flavone possessing a geranyl moiety consisting of two dimethylallyl units, as in **9**, was also isolated from this plant.

As in the case of *M. alba*, prenylated and/or prenyl moiety-cyclized flavone types of prenylated flavonoids, such as albanin A (**18**), cudraflavones B (**19**) and C (**20**), artocarpin (**21**), norartocarpin (**22**), artocarpetin A (**23**), kuwanon C (**24**), and brosimone I (**25**), have been isolated from *A. heterophyllus*, together with **4** (Baliga et al. 2011; Ko et al. 1998; Lin et al. 1996) (Fig. 5). Furthermore, phytochemical investigations have revealed that jackfruit is a rich source of highly prenylated flavones, such as artonins A (**26**) and B (**27**), cycloheterophyllin (**28**), cycloheterophyllin diacetate (**29**), and cycloheterophyllin peracetate (**30**) (Baliga et al. 2011; Ko et al. 1998; Lin et al. 1996), in which at least two dimethylallyl moieties have been cyclized with the flavone skeleton to form the pyran rings.

In contrast to *H. lupulus*, *G. glabra*, *M. alba*, and *A. heterophyllus*, 6α-hydroxypterocarpan types of prenylated isoflavonoids, such as glyceollins I–III (**31**–**33**), glyceofuran (**34**), and glyceollins IV–VI (**35**–**37**), were isolated from *G. max* as phytoalexins (Bamji and Corbitt 2017; Keen et al. 1989; Nwachukwu et al. 2013;

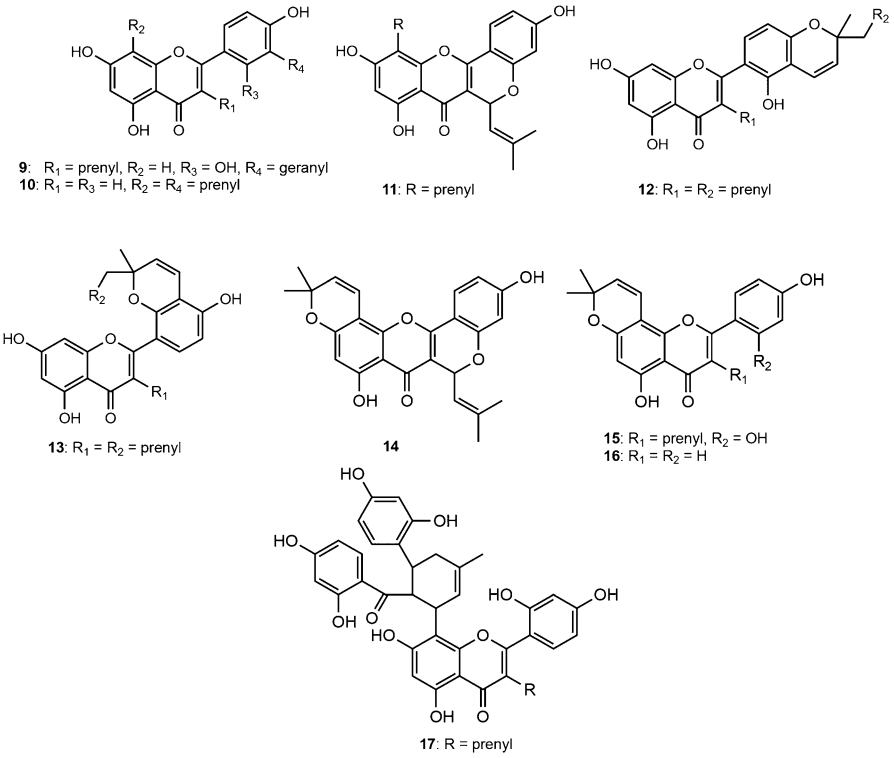

Fig. 4 Examples of bioactive dietary prenylated flavonoids (**9**–**17**) of *M. alba*

Simons et al. 2011) (Fig. 6). These are prenylated isoflavonoids with a dimethylallyl moiety at C-6 or C-8; however, most of the dimethylallyl moieties of the prenylated isoflavones from soybean have been cyclized to form pyran or furan rings.

11.3 Bioavailability and Metabolism

Dietary prenylated flavonoids possess numerous biological activities. The continuous and long-term intake of dietary prenylated flavonoids could lead to potential side effects, as alterations of their chemical structures due to our bodily metabolism lead to changes in their bioavailability, which can cause aberrant accumulation in the tissues of some organs. In general, the prenylations of flavonoids have been recognized as an important biodefense system in plants, since those modifications often enhance the bioactivities of the flavonoids over those of the non-prenylated forms and/or add new ones to the flavonoids. Recent investigations of the effects of the prenylation of flavonoids have proposed that the modification(s), which increase their lipophilicity, contribute to alterations of their bioavailability by improving the

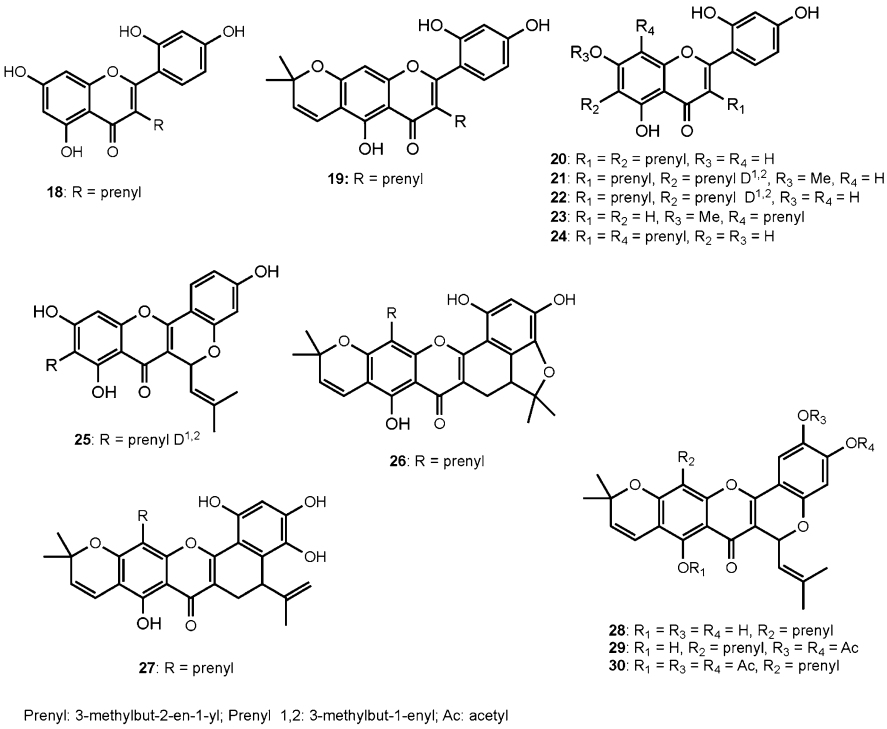

Fig. 5 Examples of bioactive dietary prenylated flavonoids (**18**–**30**) of *A. heterophyllus*

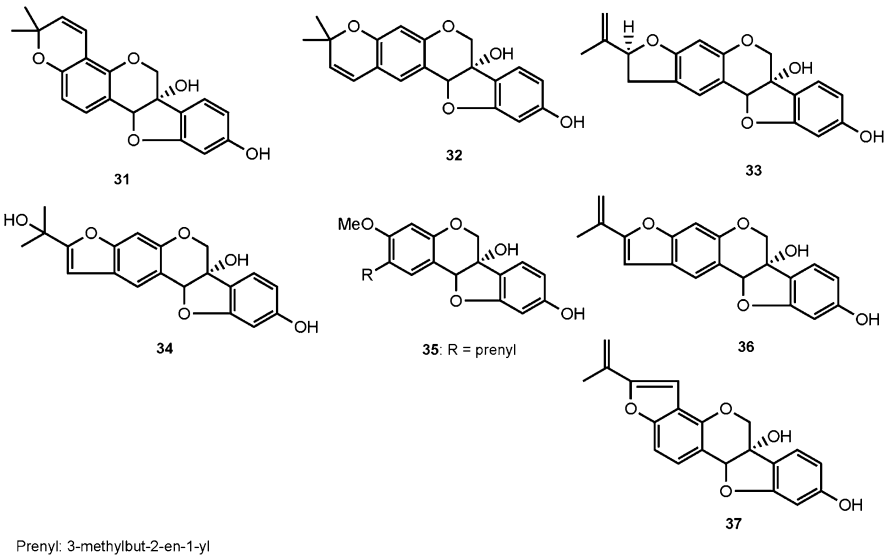

Fig. 6 Examples of bioactive dietary prenylated flavonoids (**31**–**37**) of *G. max*

absorption by tissues and/or interactions with target proteins, although further investigations will be required to understand the detailed mechanism (Mukai 2018; Terao and Mukai 2014). To be truly effective, diet-based prenylated flavonoids should be easily absorbed and metabolized. Considering the importance of these criteria, investigations have been conducted on the metabolism of dietary prenylated flavonoids from *H. lupulus*, *G. glabra*, and *G. max*.

One of the most extensively investigated diet-based prenylated flavonoids is xanthohumol (**1**), a well-known constituent of *H. lupulus*. In 2010, Hanske et al. reported the impact of human intestinal bacteria on the bioavailability of **1**, by comparing germ-free (GF) and human microbiota-associated (HMA) rats (Hanske et al. 2010). This study demonstrated that **1**, its conjugates, and isoxanthohumol (**3**) conjugates were present in the blood samples of GF and HMA rats, while **1** was found only in the blood of HMA rats. Furthermore, Hanske et al. revealed that the colonization of the rats' guts by human intestinal bacteria had no effect on the overall recovery of the applied **1** dose. However, in this study, the excretion of **1** and **3** conjugates was lower in HMA rats, and thus the study also suggested their hydrolysis by human intestinal microbiota. 8-Prenylnaringenin (**4**) was also formed by the bacterial *O*-demethylation of **3**, exclusively in HMA rats. It was recommended that, for the evaluation of the in vivo effects of **1**, the microbial impact on its metabolism in the gut should be considered (Hanske et al. 2010).

The beneficial effects on markers of metabolic syndrome in old Zucker fa/fa rats, in a rodent model of obesity, have also been reported for xanthohumol (**1**), based on a study of the in vitro phase II metabolism of **1**, using nine human recombinant UDP glucuronosyltransferases (UGT) and five sulfotransferases (SULT) (Legette et al. 2013). HPLC-DAD and HPLC-MS analyses demonstrated that **1** was significantly glucuronidated by UGT 1A8, 1A9, and 1A10. Furthermore, the study also revealed that UGT 1A1, 1A7, and 2B7 were the more important UGTs and that SULT 1A1∗2, 1A2, and 1E1 were the most active SULT forms in the sulfation reaction, while UGT 1A3, 1A4, and 1A6, as well as SULT 1A3 and 2A1, were less important UGTs for the conjugation of **1** (Ruefer et al. 2005). An in vitro investigation of the metabolism of **1** and isoxanthohumol (**3**) in human liver microsomes indicated that the hydroxylation of the prenyl methyl groups of **1** and **3** was the principal route in the oxidative process, to afford either the *cis* or *trans* hydroxylated metabolites of **1** and the *trans* isomer of **3** (Figs. 7 and 8). The investigation also revealed that the epoxidation of the double bond on the prenyl groups of both **1** and **3** led to the formation of cyclized metabolites by intramolecular reactions with the neighboring hydroxyl group (Nikolic et al. 2005). This study also showed that 8-prenylnaringenin (**4**), a potent phytoestrogen, was obtained as a demethylation product of **3**, while the similar demethylation reaction was not observed for **1**. Knowing that the cyclization of **1** to **3** could occur by acid catalysis in the stomach, **1** might contribute to the in vivo levels of the estrogenic **4**, resulting from the consumption of hops (Nikolic et al. 2005)

Very little information about the bioavailability and metabolism is available for the *G. glabra* prenylated flavonoids. Nonetheless, LC/SRM-MS analyses of rats orally administered a licorice water decoction, at 0.9 g crude drug/kg, detected the

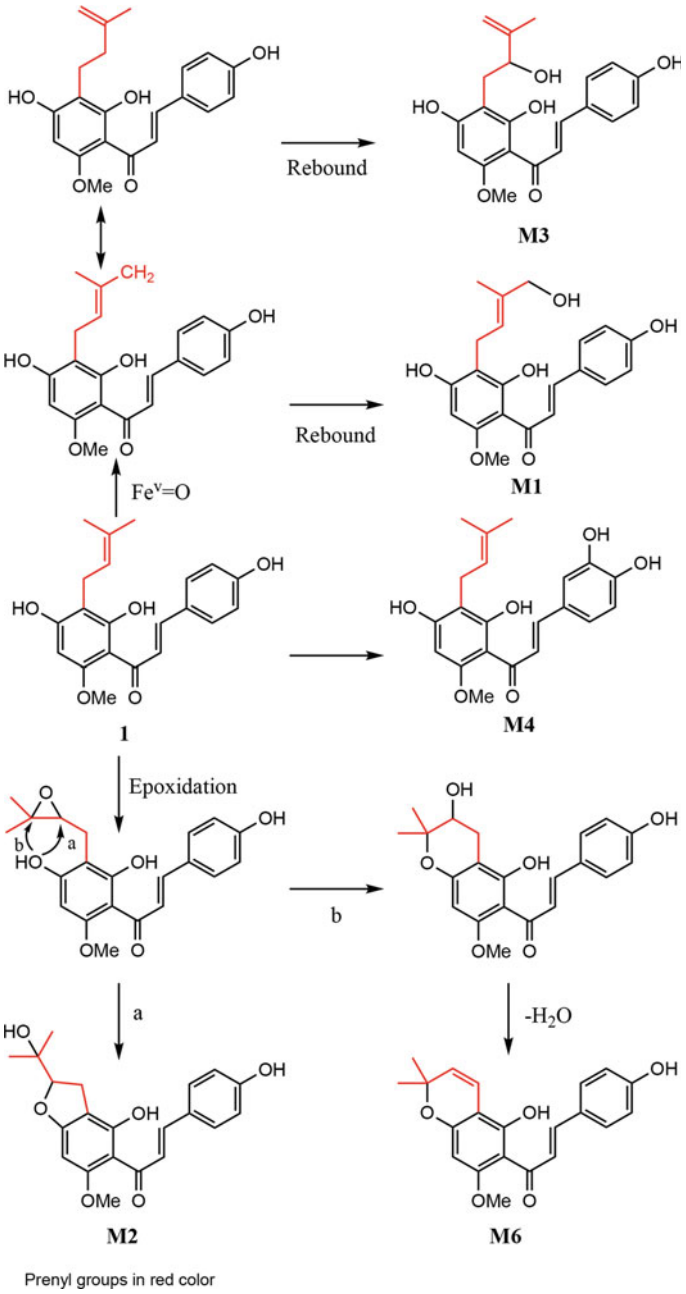

Fig. 7 Metabolic pathways of **1** catalyzed by human liver microsomes. M1 is formed by hydrogen abstraction followed by oxygen rebound. The metabolites of **3**, M7, M8, M10 and M11, are formed via analogous mechanisms (See Fig. 8). Epoxidation of the double bond leads to the formation of cyclized products by intramolecular attack of the hydroxyl group

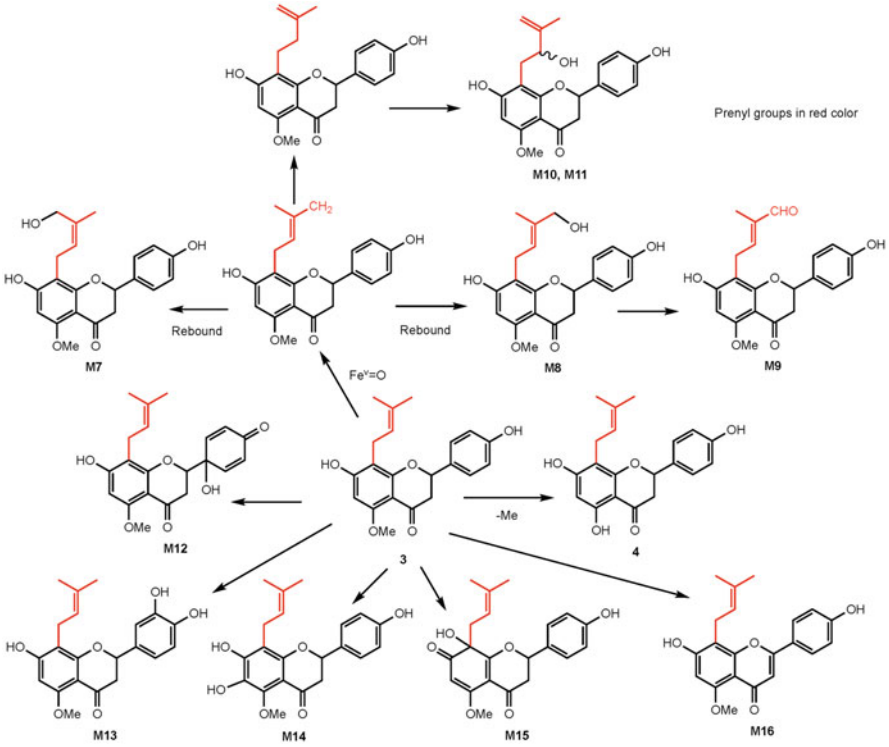

Fig. 8 Metabolic pathways of **3** catalyzed by human liver microsomes. Metabolites originate through the transformation of either the prenyl group or the flavanone skeleton. The metabolism of **3** is similar to that of **4**, except for the formation of M14 and M15. The demethylation reaction is biologically significant, since it converts the weakly estrogenic **3** into the potent phytoestrogen **4**

presence of licoisoflavone A-*O*-GluA and licoisoflavone B-*O*-GluA, together with monoglucuronide and diglucuronide conjugates of licoisoflavones A and B, in their blood, urine, and feces (Xiang et al. 2011). Isoangustone A (**8**) was also found to be metabolized in a similar manner as licoisoflavones A and B (Xiang et al. 2011). Glabridin, one of the major prenylated isoflavones in licorice, was also detected by LC–MS^n in an extract from human plasma obtained by solid-phase extraction (Aoki et al. 2005).

During an investigation of the estrogen-modulating effects of glyceollin-enriched soy protein in a postmenopausal primate model, the bioavailability of dietary glyceollins I–III (**31**–**33**) was reported in the serum (Wood et al. 2006). Interestingly, the bioavailability of single-dose prenylated flavonoids was lower than that of the parent flavonoids, despite the higher uptake into the epithelial cells of the digestive tract. This observation has been explained to be due to the restriction of the efflux from epithelial cells to the blood circulation by the prenyl groups, thus generating an insufficient increase in the plasma concentration (Terao and Mukai 2014).

Prenylation also reportedly accelerates the accumulation of quercetin and naringenin in liver and muscle tissues, respectively, after long-term feeding. In these cases, the prenyl groups would restrict the efflux from hepatocytes to the blood and enterohepatic circulations, by decreasing the excretion of prenylated flavonoids from the blood circulation and the efficient uptake by tissues. These investigations suggested that in cases of long-term supplementation, the hepatotoxicity as well as other deleterious and beneficial effects should be considered to avoid high accumulation in some tissues (Mukai et al. 2012; Terao and Mukai 2014).

The effect of prenylation on the physiological functions of dietary flavonoids was clarified by comparing the efficacy of 8-prenylnaringenin (**4**) with that of naringenin in the prevention of disuse muscle atrophy, in a model of denervation in mice (Mukai et al. 2012). The consumption of **4**, without the presence of naringenin, prevented the loss of weight in the gastrocnemius muscle, with tenfold higher potency than that of naringenin, suggesting that the prenylation generated novel functions for **4**.

Currently, there are no reports on the bioavailability and metabolism of prenylated flavonoids from *M. alba* and *A. heterophyllus*. Considering the importance of the bioavailability and metabolism of dietary prenylated flavonoids, in-depth studies should also be performed on the prenylated flavonoids from *M. alba* and *A. heterophyllus*, to provide further insights into the beneficial nutritional values of dietary prenylated flavonoids.

11.4 Bioactivities of Dietary Prenylated Flavonoids

Due to the presence of prenylated flavonoids in many food sources, their biological activities have been intensely investigated, and antioxidant, estrogenic, anticancer, anti-inflammatory, antibacterial, antivirus, and antiphospholipase phosphorylation activities have been described (Mukai 2018; Yang et al. 2015). However, these investigations have mainly been performed in vitro. Considering the relevance of in vivo studies of the dietary aspects of prenylated flavonoids, this chapter focuses on only the in vivo bioactivities of prenylated flavonoids, as well as extracts that are rich in prenylated flavonoids.

11.4.1 Extracts Rich in Prenylated Flavonoids

11.4.1.1 Menopausal Alleviation and Other Activities of Hop Extracts Rich in Prenylated Flavonoids

The efficacy of the hop has been investigated by orally administering hop extract and chemically standardized hop extracts with xanthohumol (**1**), isoxanthohumol (**3**), 8-prenylnaringenin (**4**), and 6-prenylnaringenin (**5**) to female Wistar rats, at a dose of 60 mg/kg body weight/day for 8 weeks (Keiler et al. 2017). This study indicated that the hop extracts significantly decreased the number of osteoclasts in the tibial metaphysis and prevented the reduction of the trabecular thickness from

estradiol depletion, without showing either proliferative or uterotrophic effects on the endometrium. Nevertheless, an unexpectedly high serum level of **4** relative to those of the other major marker compounds was also observed.

A xanthohumol (**1**)-rich (*c*. 18%) hop extract reportedly reduced obesity occurrence in rats on a high-fat diet (Yui et al. 2014). In this study, lower liver weights, as well as triacylglycerol levels, in the plasma and liver were also observed. These observations probably occurred from the modulation of hepatic fatty acid synthesis and the secretion of adiponectin induced by the dietary **1**-rich hop extract. The inhibition of intestinal triacylglycerol absorption by the dietary **1**-rich hop extracts also points to the reduction of triacylglycerol levels in the plasma and liver.

11.4.2 Main Bioactive Compounds

11.4.2.1 Antidiabetic Activity

The effect of the xanthohumol (**1**)-enriched stout beer consumption on the hepatic glucolipid metabolism imbalance seen in type 1 diabetes was investigated with five groups of Wistar rats, streptozotocin-induced diabetic rats given untreated drinking water and drinking treated with 5% ethanol, stout beer, and stout beer supplemented with 10 mg of **1**/L, in addition to healthy rats' untreated drinking water as a control (Lima-Fontes et al. 2017). This investigation revealed that **1** reduced hepatic fibrosis, reversed hepatic glucose transporter expression as well as glycogen content, and modulated the lipid metabolism in type 1 diabetic rats. However, **1** probably interfered with the insulin signaling pathway, as its consumption targeted the metabolic changes induced by diabetes. Further investigation of the effects of xanthohumol (**1**) and **4** on liver and skeletal muscle lipids and glycolytic metabolism in a type 2 diabetes mellitus mice model also revealed that the consumption of **1** and **4** protected against the development of type 2 diabetes mellitus metabolic-related complications and had beneficial effects by reducing obesity, preventing insulin resistance, and modulating lipid and glucose metabolic pathways (Costa et al. 2017). This showed a potential dietary approach for avoiding common metabolic illnesses, such as type 2 diabetes mellitus.

The antidiabetic action of fermented soybeans containing glyceollins I–III (**31**–**33**) in type 2 diabetic animals was investigated, by comparing the effects in diabetic mice induced by intraperitoneal injections of streptozotocin at a dose of 20 mg/kg/body weight, with those treated with no soybeans (control), 10% unfermented soybeans, and 10% fermented soybeans containing glyceollins (Park et al. 2012). Park et al. thus provided insight into not only the improvement of glucose homeostasis but also the partial enhancement of hepatic insulin sensitivity in type 2 diabetic mice by fermented soybeans containing glyceollins. In addition, the oral administration of **31**–**33** at a dose of 30 or 90 mg/kg to prediabetic rats revealed that **31**–**33** significantly decreased the blood glucose excursion and were absorbed in plasma cells 4 h after oral administration. The glucose uptake, basally stimulating insulin, was increased 1.5-fold in 3 T3-L1 adipocytes by 5 μM of **31**–**33** in a dose-responsive manner, while the glucose uptake was potentially improved (Boué et al.

2012). These findings suggested the potential benefits of glyceollins as an intervention in prediabetic conditions, as well as a treatment for type 1 and type 2 diabetes, by increasing both the insulin-mediated and insulin-independent glucose uptake by adipocytes.

11.4.2.2 Anticancer Activity

Some studies have reported the in vivo anticancer activities of xanthohumol (**1**), the most abundant prenylated chalcone in *H. lupulus* (Jiang et al. 2018). A previous investigation indicated that the central necrosis within tumor tissues, the focal proliferation areas, the reduction in inflammatory cell number, the decrease in the microvessel density, and the increase in the percentage of apoptotic cells were caused by the oral administration of **1** to nude mice inoculated with MCF7 cells (Monteiro et al. 2008). Vene et al. also reported that **1** delayed tumor progression and inhibited the growth of poorly differentiated prostate carcinoma, in transgenic adenocarcinoma of the mouse prostate (TRAMP) transgenic mice in vivo (Vene et al. 2012), while Jiang et al. reported that **1** suppressed pancreatic cancer growth in xenograft mouse models, by investigating both the tumor weight and volume in nude mice with subcutaneously implanted PANC-1 cells, followed by the administration of **1** at a dose of 25 mg/kg/day per mouse (Jiang et al. 2015). Interestingly, **1** has also been found to significantly increase animal life span by delaying the insurgence of neurological disorders due to leukemic cell dissemination, which was investigated by administering 50 mg/mouse (5 days/week) of **1** to mice by an intraperitoneal route (Benelli et al. 2012). Furthermore, Dokduang et al. suggested that **1** inhibits STAT3 activation both in vivo and in vitro, due to the suppression of the Akt-NFκB signaling pathway, and may be considered as a possible therapeutic agent against cholangiocarcinoma (Dokduang et al. 2016), because the oral administration of 50 μM of **1** in drinking water to nude mice inoculated with cholangiocarcinoma inhibited tumor growth. In addition, the suppression of triple-negative breast tumor growth in vivo by glyceollins I–III (**31**–**33**) was determined by evaluating the tumor growth in immunocompromised female nude mice with fat pads injected with either MDA-MB-231 or MDA-MB-468 cells mixed with a reduced growth factor Matrigel, followed by a treatment with glyceollins at a dose of 50 mg/kg/day (Rhodes et al. 2012).

A study of the effect of glyceollins I–III (**31**–**33**) on the growth of estrogen-dependent MCF-7 breast cancer and BG-1 ovarian cancer cells implanted in ovariectomized athymic mice revealed that **31**–**33** inhibit the estradiol-stimulated tumor growth of MCF-7 and BG-1 cells in these mice by −53.4% and − 73.1%, respectively. Thus, Salvo et al. suggested that **31**–**33** are useful antiestrogenic agents in the prevention or treatment of breast and ovarian carcinomas (Salvo et al. 2006).

11.4.2.3 Other Activities

In addition to the antidiabetic and anticancer effects, Ferk et al. reported that xanthohumol (**1**) might prevent heterocyclic mutagenesis inducing preneoplastic lesions and DNA damage in the liver and colon (Ferk et al. 2010). Furthermore, the effect of **1** on the prevention of acute hepatic injury induced by carbon

tetrachloride (CCl_4) was suggested by the facts that pretreatment of rats with **1** significantly decreased the liver weight in CCl_4-intoxicated rats, by normalizing the increased values of plasma lactate dehydrogenase, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase activities, and reduced the incidence of histopathological alterations produced by CCl_4. In addition, **1** is reportedly a good inhibitor of lipid peroxidation and protects antioxidant enzymes from the degradation induced by CCl_4 intoxication (Pinto et al. 2012).

The potential anti-inflammatory effect of glyceollins I–III (**31**–**33**) has been proposed, based on their significant reduction of 12-*O*-tetradecanoylphorbol-13-acetate-induced skin inflammation in an animal model (Kim et al. 2011). The allergic airway inflammation in a murine model of asthma was reportedly attenuated by licochalcone A (**7**) at a dose of 50 mg/kg, while **7** also prevented the increase of T-helper type 2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, and decreased the serum levels of ovalbumin-specific immunoglobulin IgE and IgG (Chu et al. 2013).

Cycloheterophyllin (**28**) and artonins A (**26**) and B (**27**) from *A. heterophyllus* reportedly possess antioxidant activities, by inhibiting iron-induced lipid peroxidation in a rat brain homogenate (Ko et al. 1998). The potent inhibition of arachidonic acid-induced platelet aggregation by **28** was also reported (Lin et al. 1996).

The most potent phytoestrogen, 8-prenylnaringenin (**4**), protected from ovariectomy-induced bone loss and had minimal trophic effects (dose independent) on the uterus and endometrium with a tenfold lower stimulatory effect than 17β-estradiol, in adult ovariectomized rats as an animal model that mimics hormone-dependent osteoporosis in menopausal women (Hümpel et al. 2005). This showed that **4** may be an alternative new candidate for the treatment of peri- and postmenopausal symptoms. In addition, **4** has been administered orally, to assess its effects on ovariectomy and long-term treatment on pituitary and liver functions in rats, at doses of 6.8 and 68.4 mg/kg body weight, and compared to treatments with 17β-estradiol benzoate (E2B) at 0.17 and 0.7 mg/kg body weight (Böttner et al. 2008). As a result, the high dose of **4** reduced the body weight gain and raised LDL levels caused by the ovariectomy effect and elicited an increase in the uterine wet weight, while the serum GH levels and IGF-1 concentrations were, respectively, increased and decreased, with an attenuation of the total cholesterol augmentation owing to the ovariectomy induction. Bowe et al. reported that the tail skin temperature was significantly reduced within 2 days of daily subcutaneous administrations of **4** at the dose of 400 μg/kg, using a rat model of menopausal hot flushes (Bowe et al. 2006). Furthermore, they also revealed that the subcutaneous co-administration of either **4** or 17β-estradiol with the estrogen receptor (ER) antagonist, ICI 182,780 (200 μg/kg), which is thought not to cross the blood-brain barrier, totally blocked the effects of **4** or 17β-estradiol on the tail skin temperature. The increased tail skin temperature in ovariectomized rats was significantly inverted by the ERα- and ERβ-specific agonists, 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol and 2,3-bis (4-hydroxyphenyl)-propionitrile, at doses of 100 and 60 μg/kg, respectively. Thus, Bowe et al. proposed that estrogens and phytoestrogens regulate the vasomotor by controlling the peripheral mechanisms related to both ERα and ERβ. On a related

note, ovariectomized rats fed with soy-free chow containing **4** for 3 months were reported to possess increased uterine weights and histologic estrogen-induced features, at a dose of 68.42 mg/kg body weight (Rimoldi et al. 2006).

11.5 Benefits

11.5.1 Diabetes

Dietary prenylated flavonoids and the extracts from their sources are used as food supplements with some beneficial effects for humans. Nevertheless, as far as we are aware, no human studies have been reported regarding the benefits of dietary prenylated flavonoids on diabetes.

11.5.2 Symptoms of Menopause in Women

The beneficial effects of the standardized hop extracts and the prenylated flavonoid, 8-prenylnaringenin (**4**), in women's menopause symptoms have been clinically investigated, and **4** reportedly possessed positive effects in postmenopausal women and could be used as an alternative to the conventional hormone replacement therapy treatment regimens (Rad et al. 2006). A study on the benefits of hop extracts on menopause symptoms attributed this property to **4** (Minecka et al. 2017). However, further studies are needed to determine the clinically effective dose of **4**.

11.6 Application in Foods

To the best of our knowledge, no specific preparation method of food rich in prenylated flavonoids has been reported so far. However, we would be obliged to comment that the food processing and products from soybean are one such example (Chen et al. 2012). Soybean processing is divided into two categories, non-fermented and fermented soyfoods. The non-fermented soyfoods include fresh soybeans, dehydrated soybeans, soy sprouts, whole fat and defatted soybeans, soy flour, and soymilk and its products, such as tofu, okara, and yuba. Fermented soyfoods are miso, natto, soy sauces, tempeh, and fermented tofu (Chen et al. 2012).

11.7 Safety: Toxicity and Side Effects

Safety for consumption has always been a primary concern for health regulators worldwide. Hence, as with any other food sources, the safety of dietary prenylated flavonoids for consumption and the side effects they may pose have also been investigated (Štulíková et al. 2018). Although the five plants discussed in this

chapter are edible, some toxicities and side effects of their chemical components have been reported.

An investigation of the effect of 8-prenylnaringenin (**4**) on the uterine wet weight of ovariectomized rats is one of the examples for the possible high risk in human applications (Keiler et al. 2015). In this study, Keiler et al. demonstrated that the subcutaneous administration of **4** to ovariectomized rats at 15 mg/kg of body weight for 3 days led to a significant increase in the uterine wet weight, as well as proliferation of the vaginal epithelium. In addition, although **4** was reported to prevent and treat hormone-dependent osteoporosis, this compound has also been found to be at least tenfold less stimulating than 17β-estradiol in the uterus of the animal model (Hümpel et al. 2005). A daily dose of 100 mg/kg body weight, with doses up to 1,000 mg/kg body weight, administered to female Sprague Dawley (SD) rats resulted in weak hepatotoxicity in the test subjects. However, there were no significant effects on the reproduction of SD rats and the development of two generations of SD rat offspring (Hussong et al. 2005).

In contrast, Keiler et al. reported that the hop extracts do not show any unwanted side effects in the mammary gland, based on the results obtained from an 8-week administration of this extract to rats (Keiler et al. 2017). Furthermore, a human study was performed with menopausal women to determine the safety and pharmacokinetics, using oral administration of hop extracts that are rich in xanthohumol (**1**), isoxanthohumol (**3**), 8-prenylnaringenin (**4**), and 6-prenylnaringenin (**5**) (van Breemen et al. 2014). Although the long half-lives of the estrogenic and pro-estrogenic effects of these prenylated flavonoids were found, no acute toxicity was reported. In addition, a recent review has also documented the safety of **1**, on the basis of experiments performed with human lung fibroblast cells (MRC-5), primary human hepatocytes, human skin fibroblasts, and oligodendroglia-derived cells (OLN-93) (Jiang et al. 2018). Furthermore, Vanhoecke et al. reported that the daily administration of 23 mg/kg body of **1** for 4 weeks to mice had no observable toxicity and side effects in the bone marrow, liver, exocrine pancreas, kidneys, muscles, thyroid, and ovaries (Vanhoecke et al. 2005). However, it should be noted that the in vivo studies were performed with normal animals to evaluate the safety and effectiveness. Hence, further investigations are required for the evaluation of the toxicity of **1** to normal organs in tumor-bearing mice (Jiang et al. 2018).

Although the safety of dietary prenylated flavonoids for consumption is a primary concern, that of the prenylated flavonoids found in *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max* has not yet been reported. The inadequate information on the dietary prenylated flavonoids provided thus far highlights the requirement of further investigations to prove their safety for consumption.

11.8 Marketed Products

H. lupulus, *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max* are marketed as processed food. *M. alba* and *A. heterophyllus* are also marketed in their raw or dried forms as fruits.

Beer contains many prenylated flavonoids from *H. lupulus*, such as xanthohumol (**1**) and desmethylxanthohumol (**2**) and their derivatives, isoxanthohumol (**3**), 8-prenylnaringenin (**4**), and 6-prenylnaringenin (**5**) (Venturelli et al. 2016). The high and increasing consumption of beer in almost all countries around the world is due to its availability in markets and affordability to almost all people, dependent on local regulations. Beer is therefore considered as the main marketed product with hops that is rich in prenylated flavonoids (**1**–**5**).

The use of *G. glabra* as food is mainly about its utilization as a natural flavoring agent, as listed by the Council of Europe. This means that licorice could only be added to food in small quantities as a flavor, and consequently, the active principles related to its prenylated flavonoids (**6**–**8**) content should be minimal (Barnes et al. 2007).

The applications of *M. alba* in food were also reported, and the fruits are generally eaten fresh or dried and marketed in a syrup form as an alternative to honey products (Ercisli 2004).

Some dietary applications of *A. heterophyllus*, one of the sources of prenylated flavonoids (**5**, **18**–**30**), have been reported so far. The raw fruits are marketed in the forms of vegetable curries and pickles, while the ripe fruits are processed into ice creams, beverages, jam, halwa, and jelly. The seeds are also available in the market in canned, brined, and tomato sauce forms (Baliga et al. 2011).

As marketed *G. max* products, we have fresh soybeans, dehydrated soybeans, soy sprouts, whole fat and defatted soybeans, soy flour, and soymilk and its products such as tofu, okara, and yuba, miso, natto, soy sauces, and tempeh. Some of these soyfoods are consumed daily by Westerners, and the most abundant in the USA are tofu, soymilk, miso, and soy sauce. Some soyfoods have also been locally adopted in America, with marketed names such as tofu ice cream, tofu hot dogs, veggie burgers, soy flour pancakes, soymilk yogurt, and soymilk cheese (Chen et al. 2012). Soybeans subjected to some extrinsic factors, such as fermentation with fungi, UV irradiation, and physical injury, produced prenylated flavonoids called glyceollins with multifunctional health-promoting properties (Nwachukwu et al. 2013). Some of these conditions applied during the preparation processes of certain soyfoods listed above have been extensively reviewed (Chen et al. 2012) and could be considered as a significant contribution in the production of soy diets rich in glyceollins (**31**–**37**).

11.9 Patents

Some findings related to plant foods with prenylated flavonoids are protected by patents. A patent was granted on the hop extract for its use in the preparation of a medication with estrogenic properties (WO2002085393A1 2002). Another patent protects the production of novel hop extracts with increased contents of prenylated chalcones and flavones. It is related to a method for producing pharmaceutical preparations of hop extracts and their use in the prophylaxis and therapy of conditions caused by estrogen deficiency or dysregulation of sex hormone metabolism (WO2003014287A1 2003). Thus far, no patent has been registered for the prenylated flavonoids found in *G. glabra*, *M. alba*, *A. heterophyllus*, and *G. max*.

11.10 Perspectives

Regarding edible plants, there are fewer reports on prenylated flavonoids, as compared to their non-prenylated counterparts. This translates into fewer investigations performed on the benefits and health risks posed by dietary prenylated flavonoids. Considering the boost to the biological activity imparted by the prenyl moiety to non-prenylated flavonoids, both the beneficial and detrimental effects of prenylated flavonoids could be amplified when consumed. Moreover, the dietary aspect also presents the bioavailability and metabolism conundrums of prenylated flavonoids that have yet to be fully clarified. Taken together, the current knowledge of dietary prenylated flavonoids is inadequate. Hence, increased efforts to investigate the benefits and health risks posed by prenylated flavonoids are necessary.

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Anthocyanins in Food

12

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Abstract

Anthocyanins are a group of water-soluble pigments in the colorful plant world, which can be used as additives in food. Anthocyanins have a basic structure of flavonoid, which have shown outstanding antioxidative effects. The daily intake of anthocyanins is abundant, and they are inversely correlated with various kinds of chronic diseases, according to the epidemiological study. Anthocyanins have anti-inflammatory effects, could regulate lipid metabolism, ameliorate insulin resistance, and improve cholesterol efflux in foam cells, which can prevent and alleviate chronic diseases such as cardiovascular diseases, diabetes mellitus, neurosis, visual degradation, cancer, and so on. However, the bioavailability of anthocyanins was proved low, which may restrict the bioactivity of anthocyanins. Some of the studies reported that the metabolites of anthocyanins could be the active compounds to prevent diseases. The commercialized products of anthocyanins are emerging in the market these years, but their development is not enough, thereby calling for more careful and systematic studies.

Keywords

Anthocyanins · Food additive · Bioactivity · Bioavalability · Metabolites · Disease prevention · Functional food · Utilization · Product

12.1 Introduction

The plants in nature are colorful and beautiful, which are mainly related to pigments with a wide range of properties in plants. For example, chlorophyll, carotene, and lutein can make plants green, orange, and yellow. Generally, plant pigments are classified into water-soluble pigments and fat-soluble pigments. The fat-soluble pigments are mainly chlorophyll, crocin, and lycopene. Among the natural pigments, anthocyanins are the largest water-soluble group, which is widely distributed (Fig. 1).

A long time ago, people began to notice that plant juice could color clothes and food. During the Renaissance, Nehemiah Grew studied the juice of vegetable organs

Fig. 1 Anthocyanins in plant food

and proposed the concept of "red pigment," which made vegetables colorful. In 1810, *Goethe Farbenlehre* made a record of the red substance of the plant in detail. Later, with the invention of the microscope, people could observe the distribution of this "red pigment" in specific cell layers of plant organs. Since nobody had any knowledge of chemistry at that time, the colorful cell sap was considered as a class of pigments. Then, in 1835, Marquart created "anthocyanin" to name the blue pigments of cornflowers. The term anthocyanin is derived from the Greek words anthos (flower) and kyanos (blue).

From the end of the nineteenth century to the early twentieth century, researchers discussed the physiological and biochemical effects of anthocyanins in plants and proposed the theory of photoprotective function of anthocyanins. In 1891, Wheldale first identified the presence of glycosyl groups in anthocyanins. In 1905, Molish first obtained anthocyanin compounds. Then Willstatter and his co-workers laid the foundation of the chemistry of these pigments. They found that anthocyanins are glycosides of anthocyanidins, the latter being polyhydroxy and methoxy derivatives of a basic structure, 2-phenyl-benzopyrylium. In the meantime, they proposed a possible way of anthocyanins synthetization in plants. They extracted anthocyanin salts by methyl alcoholic hydrogen chloride, acetic acid, or other similar solvents and precipitated a crude, often syrupy, product with ether (Blank 1947). In 1918, Parkin printed a report to show the results of a survey of 400 species of plants. He found that anthocyanins existed in the cytoplasm. But the study ignored distributions in specific cell layers and did not list the species surveyed. Subsequently, Gertz and Wheldale conducted a larger-scale survey. They analyzed the relationship between

anthocyanin content and plant species and recorded the plant species with higher expression levels of anthocyanin.

In recent years, with the advent of high-performance liquid chromatography (HPLC), the synthesis process of anthocyanins has been basically clarified. The synthesis of anthocyanins in cells is mainly concentrated in the cytoplasm around the vacuoles, via the phenylpropionic acid synthesis pathway and the flavonoid biosynthesis pathway. Later, people have studied how anthocyanins are produced in plants. The pathway of synthesis of anthocyanins is similar to other flavonoids, mainly concentrated in the cytoplasm around the vacuole, through the phenylpropionic acid synthesis pathway and the flavonoid biosynthesis pathway. There are three processes from phenylalanine to anthocyanin. The first step is the process from phenylalanine to coumarin-coenzyme A. The second step is the process from coumarin-coenzyme A to dihydroflavonol. The third step is synthesis of various anthocyanins.

Anthocyanins are widely distributed in the cytosol of flowers, fruits, stems, leaves, and root organs of angiosperms and distributed in 27 families and 72 genera. Anthocyanins are basically derived from five types of plants:fruits, vegetables, beans, cereals, and nuts. Specific content is shown in the table below (Table 1).

These figures indicates that anthocyanins are vital components of the diet of human. In recent years, people paid more attention to the biological and medicinal properties of anthocyanins. Numerous studies have demonstrated that anthocyanins have many properties useful for the health of human such as antioxidant, anti-obesity, anti-inflammatory, anti-atherogenic and anti-aggregation, antitumor, and so on. In addition, some reports have shown that supplementation of anthocyanin mixtures reduced inflammatory markers in hypercholesterol subjects rather than individual anthocyanins. All in all, it is worthwhile to explore more properties of anthocyanins.

12.2 Bioactive Constituents

Anthocyanins are natural compounds that vest color to vegetables, fruits, and other plants. They belong to a wide distribution of phenolic compounds, collectively known as flavonoids. Anthocyanins and their derivatives have various molecular structures, and they exhibit different chemical and biological effects.

12.2.1 The Main Structures of Anthocyanidins and Anthocyanins

Anthocyanidins have a 3, 5, 7-trihydroxyl-2-phenylbenzo-pyran cation structure (Fig. 2), and it is composed of an aromatic ring A bonded to an oxygen-containing heterocyclic ring C, which is also bonded by a carbon-carbon bond to a third aromatic ring B. In general, anthocyanins can undergo four types of substitution reactions such as hydroxylation, glycosylation, methylation, and acylation. The anthocyanidins are

Table 1 Anthocyanin varieties and their contents in selected common fruits, vegetables, beverages, and wines

Crop species		Anthocyanins	Content(mg/100 g FW)
Fruits	Apple peel	Cy-3-gal, Cy-3-ara,Cy-3-glu, Cy-3-xyl	10~2160
	Elderberry	Cy-3-glu, Cy-3-sam, Cy-3-sam-5-glu, Cy-3-diglu	200~1560
	Chokeberry	Cy-3-gal, Cy-3-ara	506~1000
	Garnetberry	Cy-3-glu, Dp-3-glu, Cy-3-rut, Dp-3-rut	80~810
	Grape	Cy-3-glu, Dp-3-glu, Pn-3-glu, Pt-3-glu, Mv-3-glu, Mv-3-glu-acetate	33~751
	Bilberry	Dp-3-gal, Dp-3-ara, Dp-3-glu, Pt-3-glu, Mv-3-glu	300~600
	Vaccinium oxycoccos	Dp-3-gal, Cy-3-gal, Mv-3-gal	300~500
	Blueberry	Mv-3-gal, Dp-3-gal, Dp-3-ara, Pt-3-gal, Pt-3-ara, Mv-3-ara	60~480
	Cherry	Cy-3-glu, Cy-3-rut	4~450
	Black raspberry	Cy-3-rut, Cy-3-xyl-rut, Cy-3-glu, Cy-3-sam	76~428
	Hawthorn	Cy-3-glu, Cy-3-ara, Cy-3-gal	11~407
	Cranberry	Cy-3-gal, Cy-3-glu, Cy-3-ara, Pn-3-ara, Pn-3-gal	20~360
	Huckleberry	Cy-3-gal, Cy-3-glu, Cy-3-ara, Dp-3-gul, Dp-3ara, Mv-3-glu	180~330
	Blackcurrant	Cy-3-(2^G-xylosylrutinoside), Cy-3-sam, Cy-3-glu, Cy-3-rut	250
	Blackberry	Cy-3-glu, Cy-3-rut	90~250
	Red raspberry	Cy-3-sop, Cy-3-glu, Cy-3-rut, Cy-3-glu-rut	10~116
	Strawberry	Pg-3-glu, Cy-3-glu,	12~36
	Olive	Cy-3-rut, Cy-3-glu, Cy-3-caffeylrutionside, Cy-3-(2^G-xylosylrutinoside)	42~228(dry weight)
	Pear	Cy-3-glu, Cy-3-ara	5~10(peel)
	Fig(peel)	Cy-3-rut, Cy-3-glu	3.2~9.7
	Fig(flesh)	Cy-3-rut, Cy-3-glu	0.15~1.5
	Mulberry	Cy-3-glu, Cy-3-rut	180~s185
Vegetables	Eggplant	Dp-3-gul, Dp-3-diglu-5-gul, Dp-3-diglu-caffeic acid, Dp-3-rut	750
	Purple cabbage	Cy-3-sop-5-glu acylated with p-coumaric, ferulic, or sinapyl acids, Cy-3-sop-5-glu	25~203.26
	Carrot	Pg-3-diglu-5-gul acylated with p-coumaric, ferulic, or caffeic acids	11~60
	Potato(red)	3-caffeylferulylsophoroside-5-glu of Cy and Pn	15~45
	Onion	Cy-3-glu, Cy-3-lam	5~25
	Cauliflower	Cy-3-sop-5-glu acylated with p-coumaric or ferulic acids	4.21

(continued)

Table 1 (continued)

Crop species		Anthocyanins	Content(mg/100 g FW)
Legumes and grains	Purple corn	Cy-3-glu, Cy-3-glaPg-3-glu, Pn-3-glu, Cy-3-(6′ malonylglucoside)	1779
	Rice	Cy-3-glu, Pn-3-glu	10~493
	Wheat	Cy-3-glu	0.5~16
	Black bean	Dp-3-glu, Cy-3-glu, Dp-3-glu, Pt-3-glu	
Juice	Grenadine juice	Cy-3-glu, Cy-3,5-diglu, Dp-3-glu, Dp-3,5-diglu, Pn-3-glu, Pn-3,5-diglu	600~765
	Orange juice	Cy-3-glu, Dp-3-glu	200 mg/100 mL
Fruit wine	Red wine	Cy-3-glu, Dp-3-glu, Pn-3-glu, Pt-3-glu, Mv-3-glu	9~40

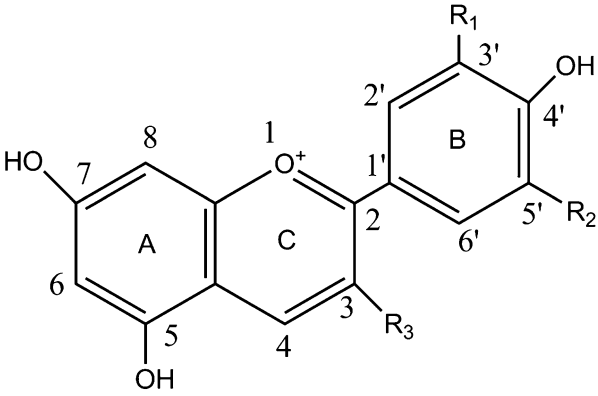

Fig. 2 Structures of the six main anthocyanidins in nature. Pg, $R_1 = H$, $R_2 = H$; Cy, $R_1 = OH$, $R_2 = H$; Dp, $R_1 = OH$, $R_2 = OH$; Pn, $R_1 = OCH_3$, $R_2 = H$; Pt, $R_1 = OCH_3$, $R_2 = OH$; Mv, $R_1 = OCH_3$, $R_2 = OCH_3$. R_3 = O-Glc

the basic structure of the anthocyanins. When the anthocyanidins are combined with sugar moiety in the form of their glycoside, they are called as anthocyanins. Up to now there are reports of more than 600 different anthocyanins and 23 anthocyanidins. However, only six of them are the most common, cyanidin (Cy), delphinidin (Dp), malvidin (Mv), pelargonidin (Pg), peonidin (Pn), and petunidin (Pt).

12.2.2 The Color of the Anthocyanins

Anthocyanins are the most important pigments in vascular plants; they are harmless and easily incorporated into aqueous media, making them useful as natural water-soluble colorants. They confer a wide variety of colors, touching practically all visible spectra, from orange and red through purple and bluish hue. Moreover, the color of red wine was originally associated with the presence of an group of anthocyanins. Color differences between anthocyanins are largely determined by

the pH, structure, the presence of co-pigments, etc., and the mechanism by which anthocyanins exhibit different colors is described below.

12.2.2.1 Anthocyanin Color at Different pH

The color of anthocyanins depends on the pH of the solution. This is because of the molecular structure of anthocyanins having an ionic property. At a lower pH solution (pH $<$ 3), anthocyanins appear to be red, and violet at pH 7–8; at a very high pH (pH $>$ 11), it is blue in color (Figs. 3 and 4).

12.2.2.2 Anthocyanin Color at Different Molecular Chemical Structures

Glycosylation

Anthocyanidin glycosylation generally reduces the maximum wavelength absorption of the anthocyanins with respect to their anthocyanidins, produces hypsochromic effect, and confers the bluish hue of the anthocyanins. Glycosylation sites and glycosylic numbers are important reasons for the anthocyanin hue and intensity.

The glycosylation site is closely related not only to the hues of the anthocyanins but also to the color intensity. On the one hand, the glycosylation site affects the hues of the anthocyanins. The more A-ring was glycosylated, the deeper the bluish hue of the corresponding anthocyanins. On the other hand, glycosylation at the specific sites of the anthocyanidins distinctly affects the color intensity of the anthocyanins. Glycosylations at C_3 site can produce the most intense colors of the anthocyanins. Additional 5-glycosylation decreased the hue angles of the anthocyanins and brings about a slight shift to red-purple for the anthocyanins.

The glycosyl number appears to mainly affect the hues of the anthocyanins, and the hue of anthocyanin may also be related to glycosyl type. The blue hues of the anthocyanins were deepening with the increasing number of the glycosyls attaching at the A-ring. Increasing the number of glucose units caused more yellow color for the anthocyanins. C_3 glycosylation with a di- or tri-saccharide reduced the hue angle of the anthocyanin slightly.

Hydroxylation

The anthocyanins A- and B-ring C_3, C_5, C_7 positions and C-ring $C_{3'}$, $C_{4'}$, $C_{5'}$ positions can be different degrees of hydroxylation. The hydroxylation of different parts of anthocyanins has an inconsistent effect on the color. The increase of the number of hydroxyl groups in the B-ring causes shifts the maximum absorption wavelength (λ vismax) of the anthocyanin visible region to the short-wave direction, i.e., produces hypsochromic effect and the bluish hue is enhanced. The hydroxyl group at the C-position of the C-ring causes the anthocyanins to exhibit different colors, most of the anthocyanins hydroxylated at the C_3 position are red, and the C_3 deoxyanthocyanin is yellow. Anthocyanins with hydroxyl groups at the C_4 position are mostly yellow.

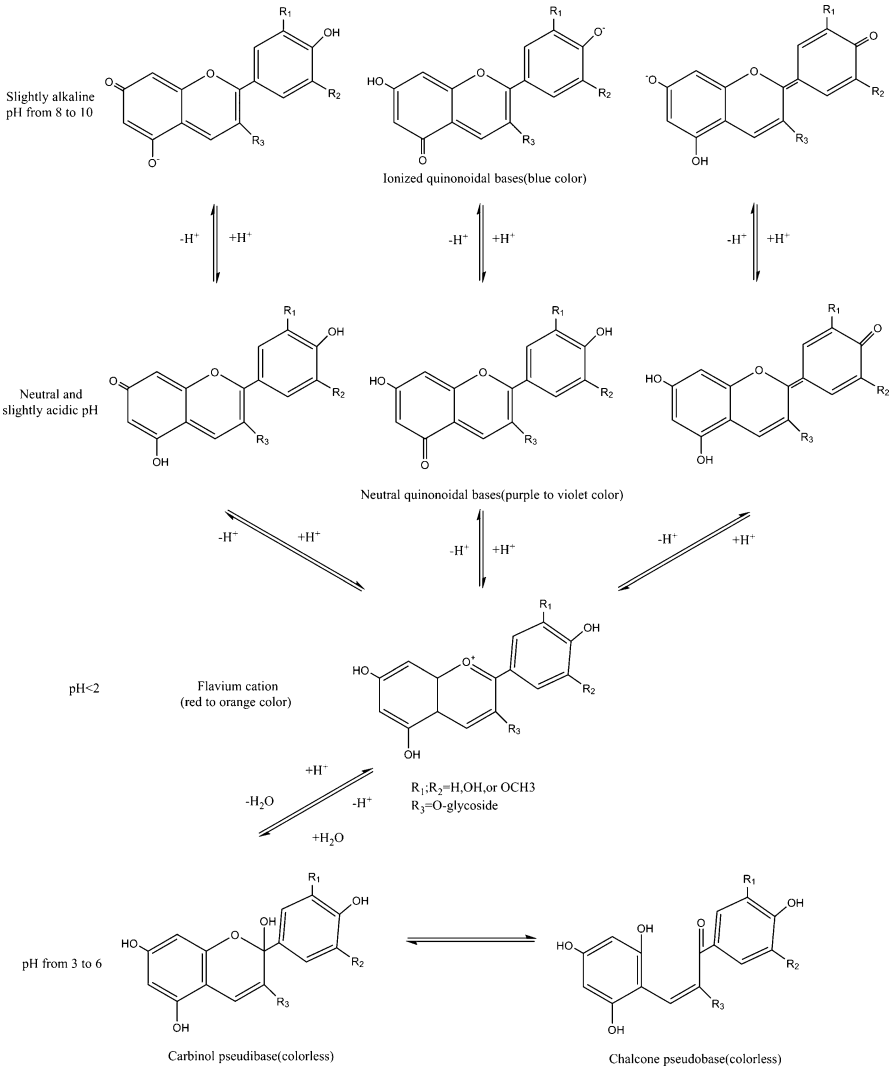

Fig. 3 Scheme of the pH-dependent structural interconversion between dominant forms of monoglycosylated anthocyanins in aqueous phase

Acylation

The acylation of anthocyanins occurs on their substituted glycosyl group and organic acids, aromatic acids, and fatty acids. Acylation produces hypsochromic effect, and the bluish hue is enhanced. However, the aliphatic series acylation of anthocyanins makes it almost no color change.

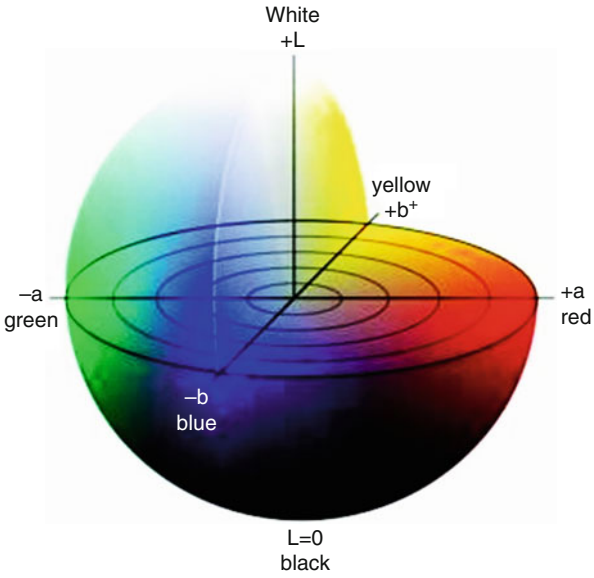

Fig. 4 The color schematic of anthocyanins (Gordillo et al. 2012)

Methoxylation

Methoxylation of anthocyanins generally occurs at the $C_{3'}$ and $C_{5'}$ positions and leads to anthocyanin yellowing, but B-ring methylation causes the anthocyanin λ vismax to move toward long waves, and the red hue is enhanced.

Co-pigmentation Effect

Co-pigmentation is a phenomenon in which pigments and other colorless organic compounds or metallic ions form molecular or complex associations, resulting in a change or an increment in the color intensity. Co-pigments are usually colorless, but when mixed with an anthocyanic solution, an interaction is carried out producing a hyperchromic effect and a bathochromic shift in the absorption spectra (UV-Vis region). The co-pigments can be flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, metals, or another anthocyanin (Castañeda-Ovando et al. 2009).

Metallic Ion Interaction

Various colors in the flower were originally explained by the formation of chelates between the metals and the flavylium salts. Anthocyanins and anthocyanidins having o-di-hydroxyl groups in the B-ring (Dp, Cy, Pt) can form metal-anthocyanin complexes. The complexation between anthocyanins and some metals such as Al, Cu, Fe, and Mg or Sn and Mo causes them to appear blue color.

12.2.3 Antioxidant Activity

The antioxidant activity of anthocyanins is largely dependent on their basic structure, such as the position and number of hydroxyl groups around the pyrone ring, the presence of acyl groups, the position and degree of methoxyl groups, and the number of sugar residues and their position. When the number of OH groups is increased, the antioxidant activity of anthocyanins is greater. The ring orientation determines the ability of anthocyanin molecule to donate hydrogen atom from a hydroxyl group to free radical. In addition, due to the positive charge of anthocyanins, the number and arrangement of aromatic hydroxyl groups, the degree of structural conjugation, and the electron-donating and electron-withdrawing substituents present in the ring structure, leading to anthocyanins, are effective donors of hydrogen. These compounds can easily provide protons to highly reactive free radicals to prevent further formation of free radicals. Among several anthocyanidins, Dp followed by Cy and Pg has been found to be the most effective in scavenging superoxide anion, whereas Pg was the most powerful against the hydroxyl radical. Regardless of the sugar attached, potency of anthocyanins toward the superoxide radical and peroxynitrite radical scavenging was in the following order: Dp > Pt > Mv ≈ Cy > Pn > Pg (Liobikas et al. 2016).

Anthocyanins can also prevent oxidation of ascorbic acid caused by metal ions through chelation of metal ions and form ascorbic (co-pigment)-metal-anthocyanin complex (Kong et al. 2003). The role of the anthocyanins can be explained by several antioxidant mechanisms, including hydrogen donation, metal chelation, and protein binding.

12.2.4 Anthocyanin Derivatives

Anthocyanin derivatives have been found in wines and beverages, or certain special types of plants, which are natural derivatives of a series of anthocyanins formed by anthocyanins during fermentation and aging, or biosynthesized in plants. Anthocyanin derivatives have high stability and exhibit different colors. Therefore, the research on anthocyanin derivatives has received more and more attention in recent years. This section mainly introduces different types of anthocyanin derivatives.

12.2.4.1 Flavanols Attached to Anthocyanin

At present, there are few studies on the direct addition of anthocyanins to flavanols to form anthocyanin derivatives. According to the reaction route, derivatives formed by direct coupling of anthocyanins and flavanols can be broadly classified into two types: A-F (anthocyanin-flavanol) or F-A (flavanol-anthocyanin) derivatives. The A-F compound is an electrophilic substitution reaction between the C_4 position of the anthocyanin cation and the C_8 or C_6 position of the flavanol to form a colorless flavene. In the formation of F-A compounds, the proanthocyanidin forms a carbocation as a nucleophile agent under acid hydrolysis. On the other hand,

cleavage of the interflavanic linkage of a flavanol oligomer provides a carbocation F^+ that reacts at C_4 position with the C_8 or C_6 of the anthocyanin, producing a colorless compound F-AOH, while F-AOH via dehydration to form the pigment in flavylium to form F-A^+ (Fig. 5).

In addition, anthocyanins can also be linked to phenolic substances via alkyl groups to form derivatives, which are generally formed by the reaction of anthocyanins, phenolic substances (such as flavanols), and small molecule aldehydes (such as acetaldehyde, propionaldehyde, etc.). In the early stages of winemaking, derivatives formed by the alkylation of anthocyanidin and flavanol play an important role in the color of the wine. The formation mechanism of the anthocyanin-alkyl-flavanol compound is under acidic conditions, small molecule aldehydes are protonated to form respective carbocations (R-HC^+OH). Then the carbocation is added to the nucleophilic site (generally at the C_8 position) of the flavanols. The formed protonated adduct that's formed then dehydrates to form a new carbocation, which is nucleophilic attack by anthocyanins to form the final anthocyanidin-alkyl-flavanol derivative.

12.2.4.2 Pyranoanthocyanins Derivatives

Pyranoanthocyanins is a series of stable anthocyanin derivatives formed during the fermentation and aging process of wine. The basic structure of pyranoanthocyanins is based on the structure of the proanthocyanins, and a cycloaddition reaction is formed between the C_4 position of the anthocyanins and the hydroxyl group at the C_5 position to form another fourth pyran ring. The pyranoanthocyanins are first discovered in wine, and a series of stable anthocyanin derivatives formed during fermentation and aging constitute different families of pyranoanthocyanins. The structure of the typical pyranoanthocyanins compounds family is shown in Fig. 6. Several typical pyranoanthocyanins families are described below.

Phenol-pyranoanthocyanins is the reaction product of grape malvidin and vinylphenol, which may be produced by cycloaddition of 4-vinylphenol, which is formed by the action of cinnamic acid decarboxylase, with anthocyanin C_4 and C_5 positions (Fig. 7).

Pyruvic acid and acetaldehyde are two common products produced by yeast metabolism during wine fermentation. These two substances interact with free anthocyanins to form a new class of pyranose pigments, vitisins, which produce a significant hypsochromically shifted.

Vitisin A is formed by cyclization of the enol structure of pyruvic acid and the C_4 and C_5 positions of dimethylcetoin-3-*O*-glucoside in an acidic environment. It is the main anthocyanin in port wine aged for 1 year.

Compared with vitisin A, vitisin B is the simplest structure of pyranoanthocyanins currently isolated from red wine, mainly produced by cyclization of acetaldehyde and malvidin (Fig. 8). Its maximum absorption wavelength is around 490 nm, showing a distinct orange-yellow characteristic in the visible spectrum.

Methyl pyranoanthocyanins is a methyl group attached to the C_{10} position of the fourth pyran ring newly formed by anthocyanins. The formation of methyl

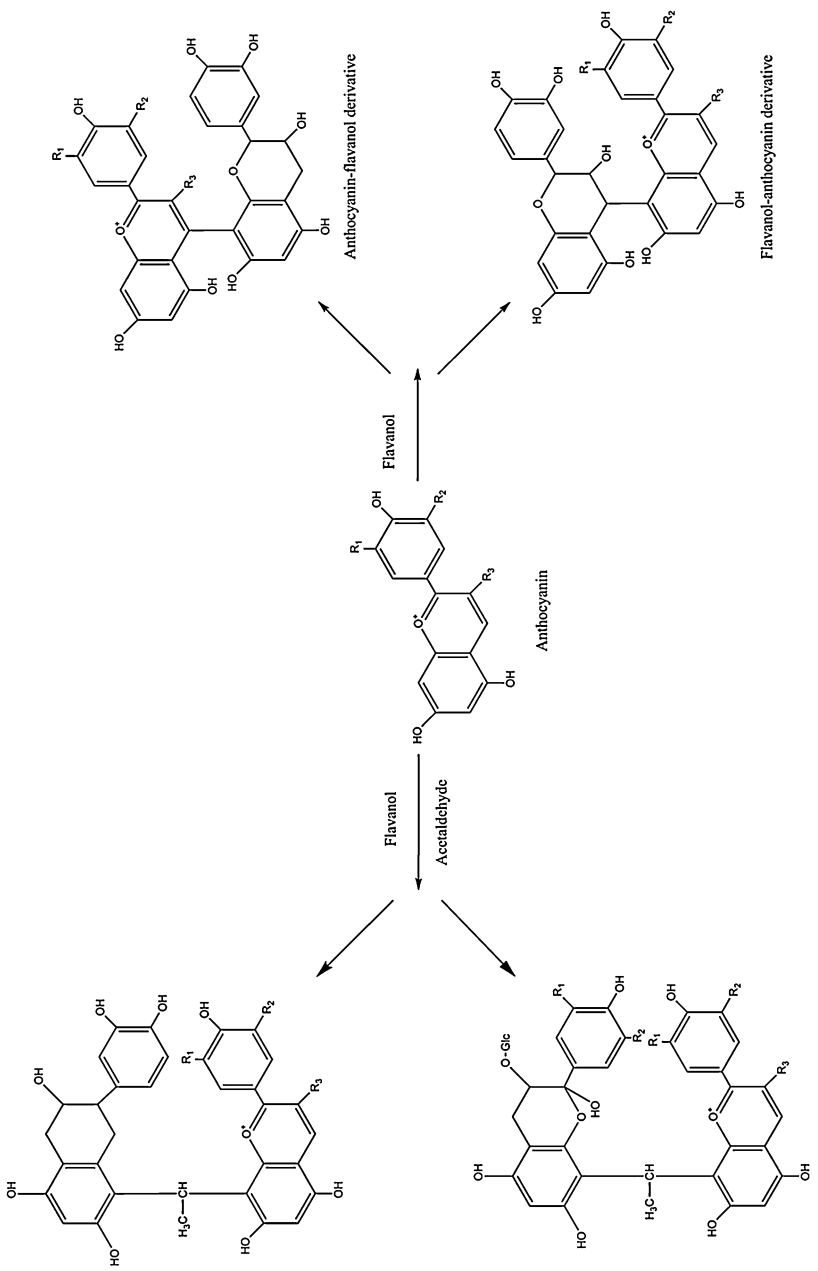

Fig. 5 Formation pathway of flavanols attached to anthocyanin

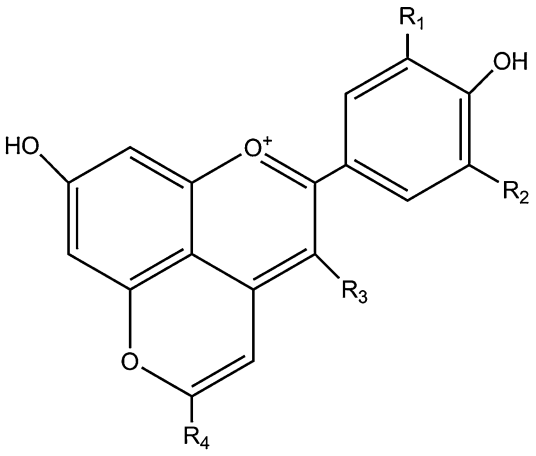

Fig. 6 The structures of pyranoanthocyanins

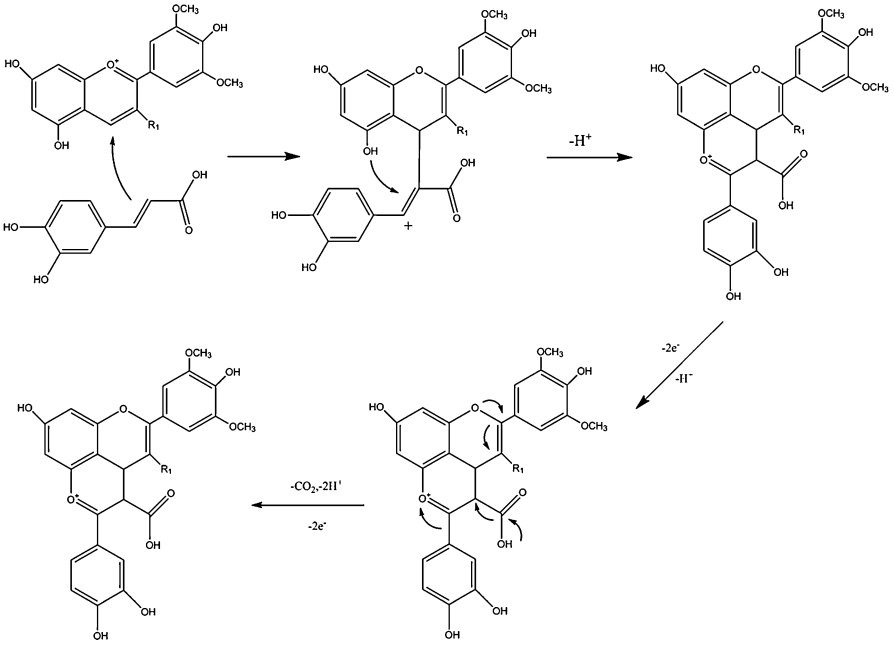

Fig. 7 Formation pathway of phenol-pyranoanthocyanins

pyranoanthocyanins in wine is derived from free anthocyanins and acetyl acetic acid reaction. Its synthetic pathway is similar to the production of vitisin A (Fig. 9). Compared with free anthocyanins, the maximum visible absorption wavelength of methyl pyranoanthocyanins is hypsochromically shifted, showing a distinct orange-yellow color in solution, and has high color stability.

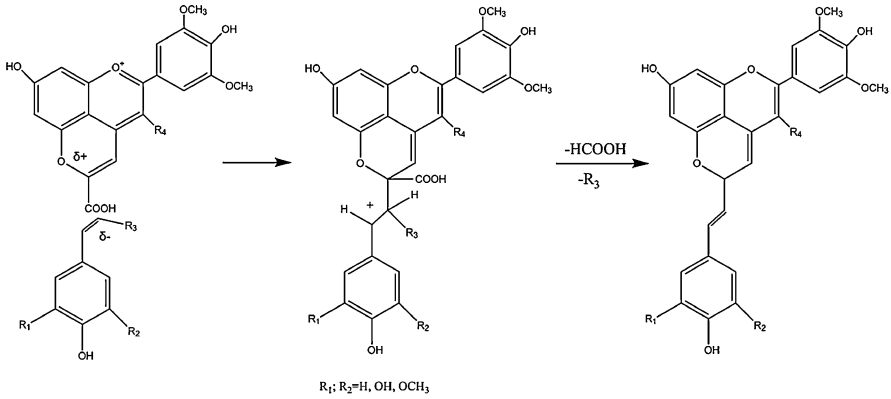

Fig. 8 Formation pathway of vitisin A

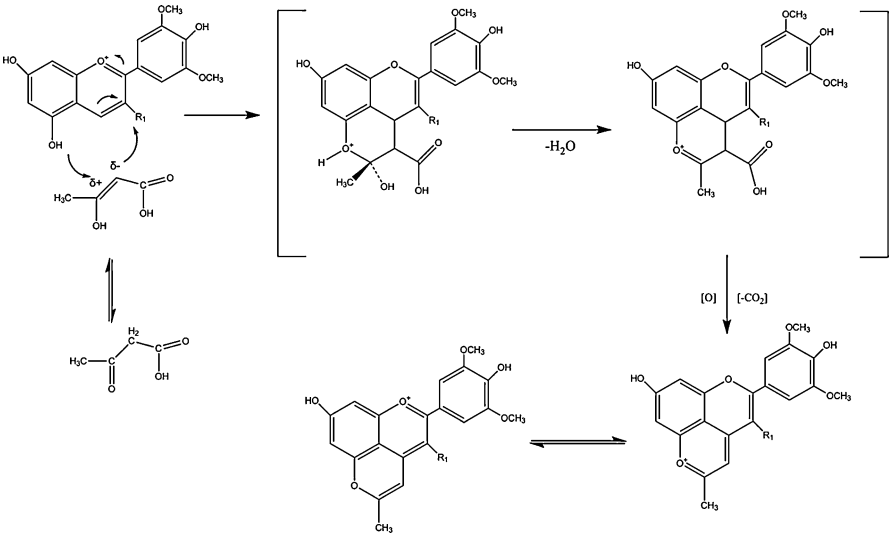

Fig. 9 Formation pathway of methyl pyranoanthocyanins

Pyranoanthocyanin-flavanols is another family of pyranoanthocyanins found in wine. The formation of pyranoanthocyanin-flavanols derivatives in wine is derived from the cycloaddition of anthocyanins and vinylflavanol; while the vinylflavanol may be derived from the cleavage of the flavanol-alkyl-flavanol polymer in the wine. Vinylflavano may also come from the intermediates that formed when flavanols and acetaldehyde are addition and dehydration reaction (Fig. 10).

The first generation of pyranoanthocyanins, such as phenol-pyranoanthocyanins, vitisin-type pyranoanthocyanins, methyl pyranoanthocyanins, and pyranoanthocyanin-flavanols, is formed by the reaction of anthocyanins and small molecule derivatives during the fermentation of fruit wine, while the first generation of pyranoanthocyanins

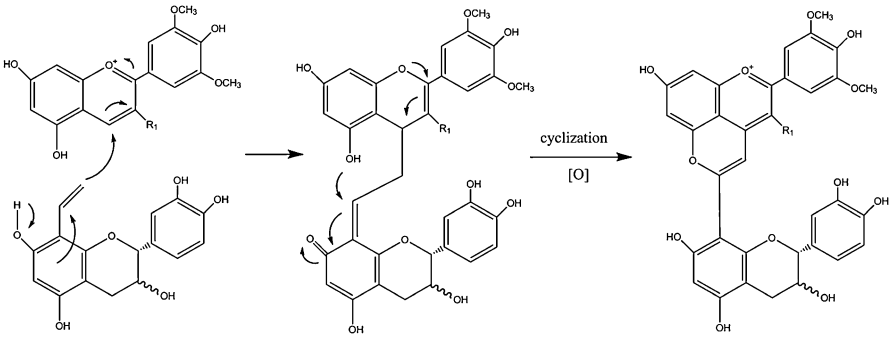

Fig. 10 Formation pathway of vinylflavanol-pyranoanthocyanins

will continue to react with small molecules to form more complex derivatives in the later stages of aging. These complex derivatives are called second-generation pyranoanthocyanin derivatives, such as portisin-type pyranoanthocyanins, pyranoanthocyanin methine dimers, and oxovitisins.

12.2.5 Anthocyanin Degradation

Different factors may affect the degradation pathway and mechanism of anthocyanins, which may affect their final products. Through the structural identification of the final product and intermediate products, it is speculated that the current possible degradation pathways for anthocyanins are mainly divided into three types. The first is that anthocyanin undergoes water addition to form methanol pseudobase glycosides and then the ring opening of C–O bonds generates chalcone glycosides and finally degrades into coumarin derivative. The second is that the anthocyanin is hydrolyzed to form a pseudobase glycoside, and further the ring open at the C–O bond to form a chalcone glycoside, which further hydrolyzes the glycosidic bond to form chalcone, and finally degrades to benzoic acid. The third is that the first hydrolysis removes glucose to form an aglycone, further producing a more unstable intermediate α-diketone, and the α-diketone finally forms an aldehyde and a benzoic acid derivative.

12.2.5.1 Thermal Degradation

The thermal degradation pathway of anthocyanins was first proposed by Markakis in 1957. He speculated that the first step in the degradation of anthocyanins in aqueous solution was the ring-opening reaction of anthocyanins and the formation of chalcone. Initial degradation step is hydrolysis of sugar moiety and aglycone formation, which forms cyclic adducts. In addition, anthocyanin would decompose upon heating into a chalcone structure, the latter being further transformed into a coumarin glucoside derivative with a loss of the B-ring (Patras et al. 2010). According to the severity and nature of heating, thermal degradation of anthocyanins

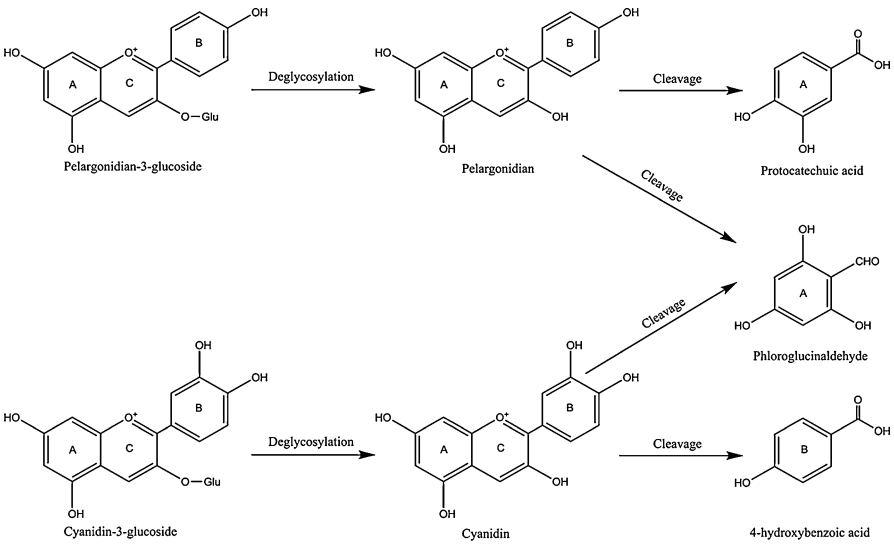

Fig. 11 Possible thermal degradation mechanism of two common anthocyanins (Patras et al. 2010)

can result in a variety of species. Figure 11 shows the degradation of anthocyanins and formation of various intermediate compounds.

12.2.5.2 Oxidative Degradation

Anthocyanins have different oxidative degradation pathways under acidic and neutral conditions. In an acidic solution of pH 1–3, H_2O_2 nucleophilic attack on the C_2 position of anthocyanins, and covalent bond cleavage between C_2 and C_3 forms benzoylphenyl acetate, the ester is easily hydrolyzed under alkaline conditions to form phenolic acids such as benzoic acid and 2,4,6-trihydroxyphenylacetic acid (Fig. 12). In a neutral solution of pH 6–7, heating causes the malvidin-3,5-diglucoside to be converted to oxime base and the oxime base to form the coumarin derivative 3,5-di-(*O*-*β*-D-glucosyl)-7-hydroxycoumarin (R_3 = *O*-glucose).

12.2.5.3 Enzymatic Degradation

Caffeic acid quinone is an oxidation product of caffeic acid, which plays an important role in the degradation of anthocyanins by polyphenol oxidase. The polyphenol oxidase degradation pathway of anthocyanins is as below: catechol is firstly oxidized by oxygen and polyphenol oxidase to form phenylhydrazine structure, and then anthocyanins are further oxidized by benzoquinone into colorless compounds. The mechanism is shown in Fig. 13.

12.2.5.4 Photodegradation

The final product of light-induced anthocyanin degradation is identical to the final product of thermal degradation; however the degradation pathways of the two are

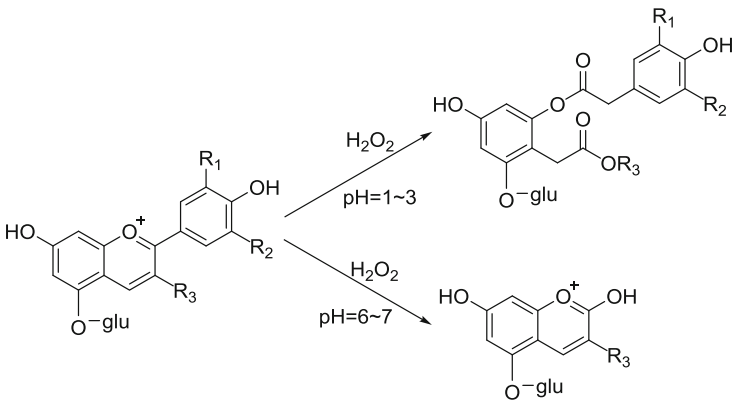

Fig. 12 Degradation mechanism of anthocyanins by hydrogen peroxide oxidation

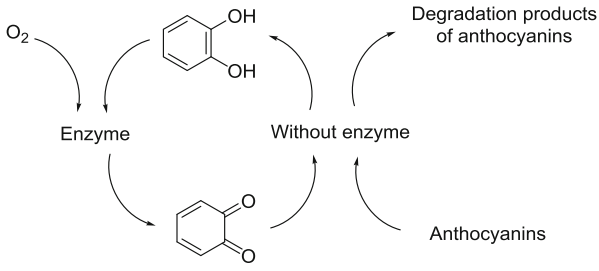

Fig. 13 Degradation mechanism of anthocyanins by polyphenol oxidases

different. The possible pathway of light-induced degradation of anthocyanins is anthocyanins are first degraded to form intermediates of C_4 hydroxyl groups, the intermediate products are hydrolyzed and opened at the C_2 position, and finally chalcone is formed and is rapidly degraded into benzoic acid and 2, 4, 6-trihydroxybenzaldehyde and other products (Fig. 14).

12.2.5.5 Pulsed Electric Field Degradation

The absorption of cyanidin-3-glucoside (Cy-3-glu) increased at 333 nm after pulsed electric field (PEF) treatment, which indicated that a chalcone compound was formed, furthermore the products 3,4-dihydroxybenzoic acid and 2,4,6-trihydroxybenzoic acid were detected. The degradation of cyanidin-3-glucoside by PEF is related to the following possible pathway: cyanidin-3-glucose first undergoes water addition reaction to form pseudobase glucoside, further opening of C–O bond to form a chalcone glycoside, the substance undergoes hydrolysis of glycosidic bonds to form chalcone, which is finally degraded to 3,4-dihydroxybenzoic acid and 2,4,6-trihydroxybenzoic acid (Fig. 15).

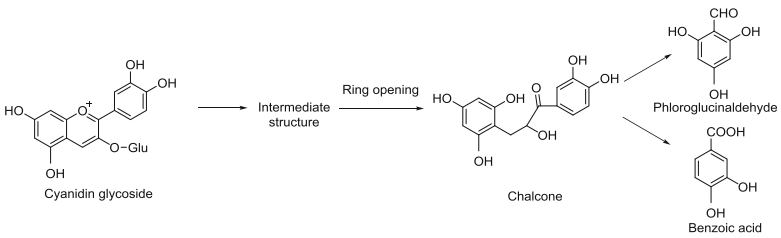

Fig. 14 Degradation mechanism of anthocyanins by light-induced degradation

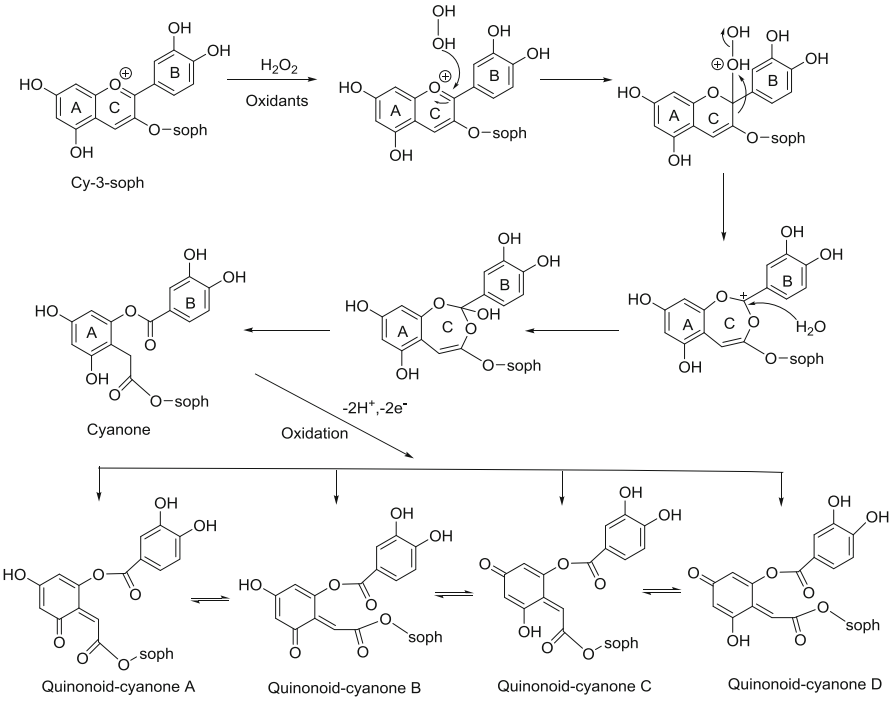

Fig. 15 One of the hypothetical schemes of Cy-3-soph degradation at pH 1.5 exposed to pulsed electric field (Sun et al. 2011)

12.3 Epidemiological Study

The anthocyanins are essential components of the human diet. They play an important role in human health as antioxidant, anti-obesity, and anti-inflammatory agent. As a result, it is crucial to associate anthocyanins intake with health effects. Due to the difference about the area of residence, seasonality, and gender, the intake of

Table 2 Dietary intake of anthocyanin/anthocyanidin from some countries

Country	Anthocyanin(mg/d)
Denmark, Odense	3–50
Finland, Helsinki	7–83
Sweden	3–35
UK	4–49
Portugal, Coimbra	34–108
Belgium, Ghent	3–33
Germany	1–63
The Netherlands, Amsterdam	3–41
Poland	3–43
Spain	29–71
Greece	37–61
France	2–61
Norway	3–43
Italy	6–93
America	2–1040
China, Guangzhou	2–69

dietary anthocyanins varies greatly among different populations. Anthocyanin intake was assessed in many epidemiological studies and clinical trial studies. Wu collected information about anthocyanins intake among different countries. Americans may consume anthocyanins ranging from 12.5 mg/d to 1040 mg/d (Wu et al. 2006). In Europe, particularly in Amsterdam, Netherlands, the dietary intake of anthocyanidins ranged from 2.8 mg/d to 25.2 mg/d. In China, anthocyanidin intake from various food ranging from 27.6 mg/d to 43.1 mg/d. Table 2 below shows the approximate intake of anthocyanins in some countries. Cyanidin-3-*O*-glucoside is used as a reference.

Bioactive compounds in foods have been gaining interest, and it is reported that botanical compounds play a protective role in reducing the risk of chronic metabolic diseases. Chronic diseases such as cancer, cardiovascular diseases (CVDs), diabetes mellitus (DM), obesity, and neurodegenerative diseases (NDGDs) are characterized by slow progression and long duration, leading to mortality and accounting for more than 60% of death in the world. As a result, more and more people are studying the properties of various ingredients of botanical compounds. Recent studies have established that regular consumption of anthocyanins could reduce risk for chronic diseases. Several cross-sectional researches confirmed a negative correlation between anthocyanin intake and incidence rate of obesity and T2DM. In a cross-sectional study of 1898 females aged 18–75 from the UK, higher intake of anthocyanins was associated with significantly lower central systolic blood pressure, pulse wave velocity, and mean arterial pressure. The ability of aortic stiffness is assessed by pulse wave velocity which is relatively well-fixed to predict the cardiovascular events (Jennings et al. 2012). In a population-based cohort study of 10,054 men and women in Finland, it was found that higher consumption of apples and berries, foods rich in

anthocyanins, was associated with a lower risk for T2DM (type 2 diabetes mellitus). Epidemiological studies also indicate that purified anthocyanin supplementation could reduce fasting blood glucose and increased serum adiponectin concentrations in patients with T2DM (Liu et al. 2014).

It is indicated that anthocyanins have many properties that play important roles in the health promotion of human such as antioxidant, anti-obesity, anti-inflammatory, anti-atherogenic and anti-aggregation, and anticancer properties, inhibiting lipid peroxidation and platelet aggregation, preventing diabetes, and so on.

12.4 Metabolism and Bioavailability

A growing number of studies have shown that anthocyanins have a number of health benefits and disease prevention effects. However, as anthocyanins are water-soluble polyphenols with strong polarity and relatively high molecular weight, they cannot actively pass through the blood vessel and are not very clear about the forms and incentives of their biological activity. This section will start with the current research to illustrate the characteristics of anthocyanins in terms of bioavailability.

We all know that anthocyanins have antioxidant, anti-inflammatory, antitumor, and other biological activities in the organism, and the absorption and metabolism of anthocyanins in the body are related to whether they can exert their biological activities well in the body. Due to the richness of anthocyanins and the complexity of digestion, so far, researches have focused on the absorption and metabolism of single anthocyanins or the overall absorption of foods containing anthocyanins. The mechanism of anthocyanin absorption and metabolism has not been fully understood.

12.4.1 The Absorption of Anthocyanins

In short, after eating an anthocyanin-rich fruit, the maximum plasma concentration can be reached within 0.5–2 h, and the maximum plasma level of total anthocyanins is in the range of 1–100 nmol/L. In animal studies, the systemic bioavailability of anthocyanins is estimated to be 0.26–1.8%. The absorbed anthocyanins are cleared from the circulation rapidly. This has led to a widespread belief that the absorption efficiency of anthocyanins is low. However, how does anthocyanin digest and absorb in the human body?

After oral administration, anthocyanins are digested and absorbed mainly in the stomach and intestines. A portion of the anthocyanins can be absorbed into the gastrointestinal wall in intact form, followed by extensive first-pass metabolism, and eventually into the systemic circulation as a metabolite. The remaining large amount of anthocyanins can enter the intestinal tract and be absorbed by the small intestine or catalyzed by intestinal microbes in the large intestine to form metabolites (Fang 2014).

12.4.1.1 The Translocation of Anthocyanins

In the mouth. There are few studies on the degradation of oral anthocyanins. The only studies have shown that anthocyanins can be taken up by oral epithelial cells in the oral cavity, and the rate of absorption is closely related to the structure of anthocyanins. In a study by Kom Kamonpatana, 12 healthy individuals evaluated the stability of anthocyanins, mucus binding, and uptake of epithelial cells after retaining red or strawberry juice in the mouth for 5 min (Kamonpatana et al. 2014). The experimental results indicate that the anthocyanin structure affects the stability and uptake of buccal cells. In the mouth, digestion of saliva exposes anthocyanins from the food matrix in preparation for absorption of the gastrointestinal tract.

In the stomach. Gastric absorption is an important event with respect to the large and rapid appearance of anthocyanins in systemic circulation. Gastric contents have a pH ranging between approximately pH 1.5 and 4 because of the influx of natural gastric secretions, including HCl and stomach enzymes. Anthocyanins can be stabilized by the low pH environment of the stomach and exist in a variety of ionic forms. However, in vivo bioavailability experiments have fully demonstrated that anthocyanins can be absorbed by the stomach wall in prototype form, and the absorption rate is faster. In general, after oral administration of anthocyanins, anthocyanins can be detected in plasma after 15–60 min, which is mainly absorbed through the stomach wall and then enters the blood (Talavéra et al. 2003). Under the action of gastric acid, the anthocyanins in the food are completely dissolved and released, and most of the anthocyanins can bind to the bilirubin translocation enzyme to promote their passage through the gastric mucosa. Therefore, its absorption speed is faster. Subsequently, anthocyanins absorbed from the stomach enter the blood and other tissues.

The small intestine. Depending on the size and composition of the meal, the food is discharged from the stomach into the small intestine within a few hours. In the intestine, lactate hydrolase hydrolyzes glycosides to form more hydrophobic anthocyanins that can passively diffuse into intestinal cells (Kay 2006). A variety of different transport mechanisms have also been proposed in this section, including sodium-dependent glucose cotransporters and other glucose-related transporters (GLUTs) or entero-biliary transposases. Once passed through the intestinal cell wall, anthocyanins may be modified by xenobiotic metabolism in the intestinal cells. The extent to which anthocyanins are affected by the first stage of metabolism is unclear.

Inside the small intestine, the relative proportion of anthocyanin forms (glycosides or aglycone, conjugated or unconjugated, and flavylium, chalcone, or hemiketals) during the first passage through the intestine at any time, and its location is impossible to predict. The proportion of phenolic acids derived from anthocyanin increases with increasing distal passage through the GIT. Enterohepatic recycling of anthocyanin adds to this complex pool of anthocyanin-derived metabolites. It can be expected that the pool of anthocyanins is increasingly more catabolized over time, and the pool of phenolic acids is increasing.

The large intestine. Like the small intestine, the large intestine constitutes a very large surface area for anthocyanin absorption. However, it has been suggested that

regions of the large and small intestine are not equivalent in their anthocyanin absorptive capacity, and, specifically, the anthocyanins are not well absorbed from the colon. It is assumed that both large and small intestinal walls can participate in the enterohepatic recycling of anthocyanin. This enterohepatic recycling appears to be extensively based on the long residence time and diverse profile of urinary anthocyanin metabolites detected by LC-MS-MRM.

The absorption diagram of anthocyanins is as follows (Fig. 16):

Anthocyanins can be absorbed intact despite having different molecular sizes and types of sugar or acylated groups attached. Many factors can affect the rate and extent of anthocyanin absorption, such as the glycone, sugar moiety, and acylated groups. The extent of absorption may be decreased for the complex anthocyanins. Digestion and absorption of anthocyanin mainly depend on the gastrointestinal tract. Therefore, it is obvious that gastrointestinal diseases such as atrophic gastritis and peptic ulcer can reduce the secretion of digestive fluid, change the pH of the intestinal tract correspondingly, and reduce the absorption utilization rate of many nutrients, which also has a certain impact on the absorption of anthocyanin. As we all know, as the age increases, many functions of the body will decline. All tissues and organs generally show a decline in function during aging. From the perspective of the digestive system, the gastrointestinal mucosa becomes thinner with age, the gastrointestinal glands and villi gradually shrink, the muscle fibers shrink, and the elasticity decreases. Studies have shown that different levels of tension and age in animals can affect the absorption and utilization of cyanidin. With the increase of age, the absorption rate of anthocyanins will also decrease accordingly, and age is also one of the influencing factors of anthocyanin absorption. Meanwhile, anthocyanins belong to the broad-based flavonoids, and their absorption is affected by the chemical substances of the same kind. Isoquercetin (quercetin 3-*O*-glucoside)

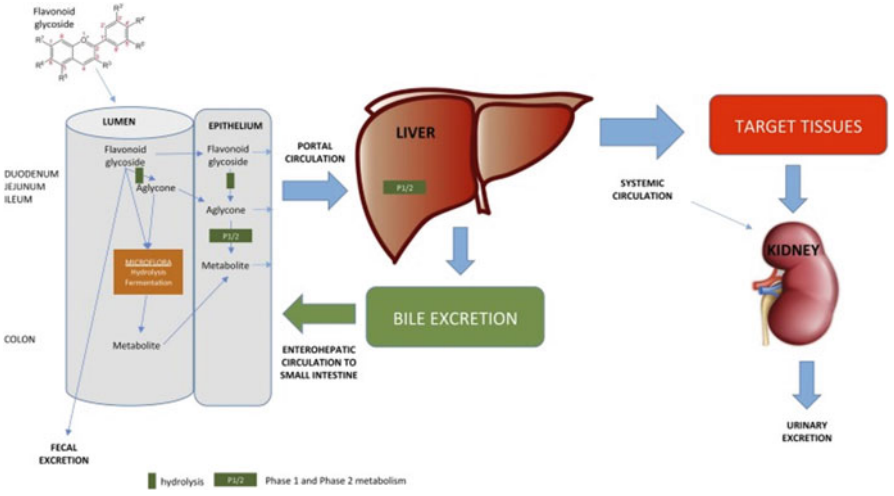

Fig. 16 Absorption of anthocyanins

has a similar chemical structure with cyclamine 3-*O*-glucoside. Studies have found that the transport and absorption of isoquercetin are affected by a protein called sodium-glucose-linked transporter in the epithelial mucosa. At the same time, this sodium-glucose-linked transporter can be inhibited by glucose and rhizoside. Therefore, anthocyanin absorption may be inhibited when foods have high glycemic index or when there are large amounts of structural analogues.

12.4.2 Anthocyanin Metabolites in Human Body

Anthocyanins are mainly absorbed in the stomach and intestines. In different organs, the molecular structure of anthocyanins changes due to environmental changes. For example, in the stomach, the pH is low and anthocyanins are present in the form of flavylium cation. However, in the intestinal tract, the pH is close to neutral, and the stability of anthocyanins is reduced, and anthocyanins are converted to colorless chalcone. Anthocyanins enter the circulatory system through the liver after being absorbed in the gastrointestinal tract. Some anthocyanins are metabolized in the liver and kidney by methylation and glucuronidation. For example, cyanidin-3-*O*-glucoside can be converted into paeonidin-3-*O*-glucoside and petunia-3-*O*-glucoside. In the eyes, cerebral cortex, and cerebellum, it is mainly present as an anthocyanin prototype.

12.4.2.1 Process of Anthocyanin Metabolism

The metabolism of anthocyanins runs through the entire digestive tract but is mainly carried out in the small intestine. Previous studies have shown that initial metabolism occurs when anthocyanins enter the mouth. For example, saliva can enhance the bioavailability of some flavonoid nitrous oxides with catechol structure (Peri et al. 2005).

In the stomach, anthocyanin maintains a 2-phenylbenzopyran structure due to the very low pH (1–2) and strong acidity of the gastric juice. In contrast to the stomach, the pH in the intestine is near neutral, the stability of anthocyanins is reduced, and anthocyanins of different structures coexist. Moreover, the intestinal flora, especially the flora of the colon, further modifies the structure of anthocyanins. Anthocyanins are unstable under neutral pH conditions, and their aglycones are cleaved by carbocyclic rings and eventually decomposed into corresponding phenolic acids and aldehydes. Studies have shown that under the action of bifidobacterium and lactobacillus, malvac-3-glucoside can be converted into lilac acid. Cyanthrin-3-glucoside was transformed into m-phenolic and protocatechuic acids. The above describes anthocyanins which are not absorbed by the stomach and small intestine and are largely modified into phenolic acids in the colon. However, the specific intestinal environment of the small intestine modified by anthocyanins is difficult to determine.

In contrast, some transformation occurs when anthocyanin is injected intravenously into the bloodstream. For example, cyanidin-3-glucoside can be converted to

peonidin-3-glucoside and delphinidin-3-glucoside, both of which continue to be converted to petunia-3-glucoside and finally to mallow-3-glucoside.

12.4.2.2 Anthocyanin Metabolites

The conversion reaction of anthocyanins in vivo mainly includes methylation, acetylation, glycoside hydrolysis, and glucuronic acid-binding reaction. The current research is not particularly clear about how these changes occur in anthocyanins. However, most studies have shown that there are protoplast anthocyanins, anthocyanin conjugates, and some phenolic acids in the blood and in some organs, of which phenolic acid is the common metabolite. And the metabolites of cyanidin-3-*O*-glucoside are shown in Fig. 17.

12.4.2.3 Factors Affecting Anthocyanin Metabolism

Different intake pathways have an important effect on the metabolism of anthocyanins. Oral intake of anthocyanins is partially metabolized by the gastrointestinal mucosa and enters the circulatory system through the liver. Some of the anthocyanins are metabolized by methylation and glucuronidation, and some metabolites enter the intestinal tract through bile; while intravenous anthocyanins are not after the liver metabolizes, it will reach various organs through blood circulation.

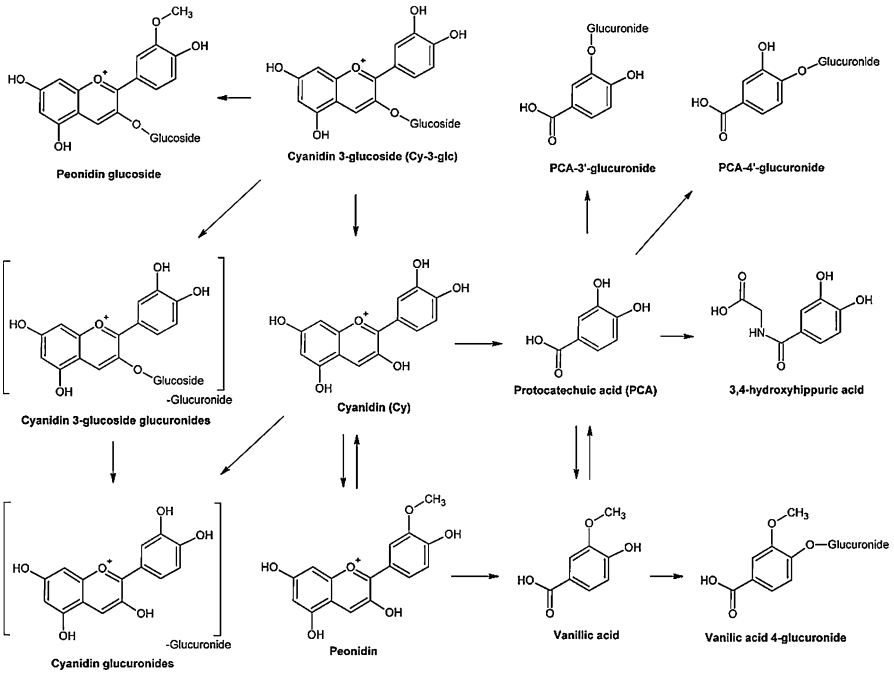

Fig. 17 Metabolites of cyanidin-3-*O*-glucoside

The amount and type of anthocyanins ingested are different, and the structure of anthocyanins modified by the intestinal flora in the intestinal tract is different, so the amount of phenolic acids produced by anthocyanins after being metabolized by intestinal microbes may also vary. The aging process is associated with reduced hepatic perfusion and morphology including reduced hepatocyte density, which has been suggested to reduce the phase I and phase II metabolism of xenobiotics and therefore potentially flavonoid metabolism.

Studies have shown that intestinal microbes deglycosinate compounds, but microorganisms can also undergo a range of other transformations, including oxidation, demethylation, and catabolism to smaller fragments, including small phenolic acids and aromatic catabolites. A wide range of microbial-derived metabolites specifically identified for anthocyanins support strong interactions between flavonoids and microbiome. Since the gut microbes of different genus act on different kinds of anthocyanins, the type and quantity of gut microbes affect this process.

12.4.3 Bioavailability of Anthocyanins

In recent years, human studies on the bioavailability of anthocyanins have also increased rapidly. Studies have shown that anthocyanins enter the blood in the form of glycosides, whether in human or animal models, and are excreted from the urine. After ingesting a certain amount of blackcurrant (including C3G 3.57 mg/kg body weight), blackcurrant proanthocyanidin can be measured in human plasma and urine. The concentration in plasma is 0.12 nmol/L, and the anthocyanin excreted in urine is estimated to be 0.11% of the intake.

In addition, these experiments have also shown that anthocyanins have extremely low bioavailability and that anthocyanins are rapidly degraded and excreted in the human body. The bioavailability of anthocyanins in red wine (2 cups/day) was 218 mg/300 mL, and after 12 h the urine content was 5% of the intake (Bitsch et al. 2004). Bub et al. studied the uptake and utilization of the main anthocyanins in red wine and red grape juice, namely, malvidin-3-*O*-glucoside. Ingested red wine (containing 68 mg malvidin-3-*O*-glucoside/500 mL) or red grape juice (containing 117 mg malvidin-3-*O*-glucoside/500 mL) can be quickly measured in plasma and urine (Bub et al. 2001). The maximum concentration and appearance time in plasma were red wine 1.4 nmol/L, 20 min, and red grape juice 2.8 nmol/L, 180 min. The amount of anthocyanin in red wine and red grape juice in urine was 0.03% of intake.

The bioavailability of anthocyanins was the subject of several excellent reviews. In contrast to the accumulating evidence supporting their health effects, the plasma concentrations of anthocyanins were found to be low. The percentage of intact anthocyanins excreted in urine was found to be $<0.1\%$ in human studies. In animal studies, absolute bioavailabilities of anthocyanins were found to be only 0.26–1.8% when intravenous administrations were used for comparisons (Table 3).

Table 3 Absorption and metabolism of anthocyanins in humans

Materials	Anthocyanin dose (total intake)	C_{max}	T_{max} (h)	Urinary excretion (%)
Blackcurrant	236 mg	0.120 umol/L	1.25–1.75	0.11(8 h)
Red wine (500 mL)	68 mg (Mv-3-glue)	0.0014 umol/L	0.8	0.02(6 h)
Dealcoholized red wine	56 mg (Mv-3-glue)	0.0017 umol/L	1.5	0.02(6 h)
Blueberry powder (100 g)	1.2 g	0.029 umol/L	4	0.003–0.012
Elderberry extract (12 g)	720 mg	0.097 umol/L	1.2	0.06(24 h)
Red wine (400 mL)	180 mg	43 ng/mL	1.5	0.23(7 h)
Red grape juice (400 mL)	284 mg	100 ng/mL	0.5	0.18(7 h)
Blackcurrant juice	1.24 g 0.72 g 0.75 g	53 ng/mL 16 ng/mL 32 ng/mL	0.75 0.75 1.5	0.07(4 h) 0.05(4 h) 0.05(4 h)
Chokeberry extract(7.1 g)	721 mg	0.096 umol/L	2.8	0.15(24 h)

C_{max}, maximal plasma concentration; T_{max}, time to reach C_{max}; urinary excretion (%), % of intake; Mv-3-glu, malvidin-3-glucoside

In summary, the bioavailability of anthocyanins has the following characteristics:

1. The bioavailability of anthocyanins is low, and the ratio of excretion in urine to oral bioassay is generally less than 0.1%.
2. Anthocyanin was absorbed rapidly, and the maximum concentration can be measured in the plasma for 15–60 min and completely eliminated within 6–8 h. This phenomenon indicates that anthocyanins are mainly absorbed through the stomach.
3. Anthocyanins are absorbed by the intact anthocyanin prototype, circulated in the circulation system, and finally discharged through the urine.

12.5 The Bioactivities of Anthocyanins

Anthocyanins have antioxidant and anti-inflammatory properties, regulate lipid metabolism, improve insulin resistance, and fight tumors.

12.5.1 The Antioxidant Activity of Anthocyanins

Reactive oxygen species (ROS) is an ion, atom, or molecular group with a single unpaired electron. Free radicals in animals are mainly oxygen free radicals,

accounting for about 95% of the total amount of free radicals. ROS include oxyanion radicals ($O_2-\cdot$), hydrogen peroxide molecules (H_2O_2), hydroxyl radicals ($OH\cdot$), hydroperoxy groups ($HO_2-\cdot$), and the like. Anthocyanins exert antioxidant capacity by directly scavenging free radicals and indirectly scavenging ROS. Oxygen anion radicals, hydroxyl radicals, and various lipid peroxidation radicals can initiate lipid peroxidation. The phenolic hydroxyl group on the molecular structure of anthocyanins has reducibility, and can release electrons by self-oxidation, directly removing various free radicals, thereby achieving the purpose of inhibiting oxidation.

Anthocyanins can reduce the level of oxidation by increasing the activity of intracellular superoxide dismutase and glutathione transferase. In addition, it can inhibit the activity of oxidases such as phospholipase and lipoxygenase which induce intracellular oxidation. When ROS accumulation in the body is excessive, Nrf-2 binds to the target gene, promotes the production of antioxidant enzymes and other antioxidants, and improves the body's antioxidant capacity. When the oxidative stress is too severe, the body's immune regulation system centered on NF-κB, AP-1, and MAPK participates in the reaction and regulates the reaction. In case of inflammation and hypoxia, the dynamic balance of the production and elimination of ROS in the body is broken, resulting in oxidative stress. When oxidative damaged DNA and protein accumulation exceed the limits of cellular repair and detoxification mechanisms, a series of diseases occur. Anthocyanins can directly enter cells to scavenge free radicals, increase the activity of antioxidant enzymes, and inhibit lipid peroxidation.

12.5.2 Anti-inflammatory

Anthocyanins have significant anti-inflammatory effects. Anthocyanins can regulate cell activation, inhibit the production of cytokines, inhibit the migration of inflammatory cells, and inhibit transcription factors to impede inflammatory factors including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and transforming growth factor-β (TGF-β), IL-8, and IL-l0. Anthocyanins can also bind to the receptors of inflammatory factors and reduce their effects.

Interestingly, anthocyanins exert anti-inflammatory effects in vivo through the following three pathways: anthocyanins have a regulatory effect on MAPK and NF-κB inflammatory signaling pathways, the regulation of anthocyanins on the inflammatory signaling pathway in CD40-CD40L, and the regulation of anthocyanins on nuclear receptor signaling pathway (Vendrame and Klimiszacas 2015). Anthocyanins inhibit the release of inflammatory factors or mediators by affecting the three pathways above.

12.5.3 Regulation of Lipid Metabolism

Long-term intake of high-fat diets easily leads to excess free radicals in the body, pulmonary lipid metabolism disorder, excessive accumulation of fat, and other

phenomena. It regulates the expression of certain adipocytokines, enzymes, and genes that control fat metabolism, inhibits fat synthesis, and promotes into fat decomposition.

It is a possible mechanism of action of anthocyanins to regulate lipid metabolism and alleviate obesity caused by high-fat diet. The effects of anthocyanins on dyslipidemia are mainly reflected in lowering serum total cholesterol, triglyceride, and low-density lipoprotein-cholesterol levels and increasing levels of high-density lipoprotein-cholesterol and apolipoprotein.

12.5.4 Amelioration of Anthocyanins on Insulin Resistance

Insulin resistance (IR) means that insulin in the body promotes the ability of organs, tissues, and cells to absorb and utilize glucose. In order to maintain relatively normal blood sugar levels, the body must compensate for the secretion of insulin and form hyperinsulinemia. IR refers to the decrease in the efficiency of insulin-promoting glucose uptake and utilization by various reasons, and the compensatory secretion of excess insulin produces hyperinsulinemia to maintain the blood glucose. Insulin resistance easily causes metabolic syndrome and type 2 diabetes.

Interestingly, anthocyanins can use their own phenolic hydroxyl groups to neutralize excess ROS in cells, reduce oxidative stress levels, and prevent free radical damage and secondary insulin resistance; on the other hand, by inhibiting stress activation of stress proteins such as JNK and MAPK in adipocytes to increase tyrosine phosphorylation of IRS-1 after insulin stimulation and GLUT4 from cytoplasm to cell membrane to improve insulin sensitivity in liver and muscle tissue, anthocyanins can also improve the exocrine function of adipose tissue by regulating gene expression of human adipokines, thereby improving insulin resistance status of polar bodies.

12.5.5 Antitumor Effect of Anthocyanins

In the initial stage of tumor, anthocyanins can protect DNA from genetic mutation by anti-oxidation; in the tumor stage, anthocyanins can reduce the expression of inflammatory factors, such as COX and NO by inhibiting the MAPK signaling pathway, and inhibit VEGF. Its expression controls the proliferation of tumor cells; in the development stage of tumors, anthocyanins exert anticancer effects by activating JNK and caspase-3 to promote apoptosis of cancer cells and inhibiting MMP expression to prevent invasion and metastasis of cancer cells.

12.6 Anthocyanin's Benefits in Human

Anthocyanins are glycoside derivatives of anthocyanidin, which belongs to flavonoids. Anthocyanins are found in a wide variety of plant foods such as blueberries, cherries, purple potatoes, and black rice and very easy to obtain. Furthermore,

anthocyanins have various bioactivities such as scavenging free radicals, cancer prevention, inflammatory suppression, inhibiting lipid peroxidation, preventing diabetes, losing weight, and protecting vision, and they can protect cells and animals because of these bioactivities. In addition, human studies also manifest that anthocyanins play an important role in protecting human diseases including metabolic-related diseases and non-metabolic-related diseases.

12.6.1 Protective Effects of Anthocyanin on Cardiovascular Disease

Cardiovascular diseases (CVDs) are manifestations of general vascular disease or systemic vascular disease in the heart. The causes can induce CVDs including atherosclerosis, arteritis, hypertension, hyperlipidemia, leukemia, anemia, thrombocytopenia, and so on. CVDs are a common disease that seriously threatens the health of humans, especially middle-aged and older people over the age of 50. And they are characterized by high prevalence, high disability, and high mortality. According to reports, approximately 85.6 million ($>1/3$) of American adults have CVD, and among them, 43.7 million people are ≥ 60 years old. And people all over the world spend a plenty of money to treat cardiovascular diseases. We should pay attention and focus on the prevention of CVDs. Recently, several large cohort studies, follow-up studies, and cross-sectional studies have shown that anthocyanin intake is negatively correlated with cardiovascular disease incidence.

The studies further confirm that anthocyanins can reduce the incidence and mortality of atherosclerosis, coronary artery disease, hypertension, nonfatal myocardial infarction, and stroke. Total cholesterol and serum triglycerides are routine projects for lipid analysis and represent risk factors for the predisposition of CVD. Anthocyanins could decrease the level of total cholesterol and serum triglycerides. Low-density lipoprotein (LDL) is a lipoprotein particle that carries cholesterol into peripheral tissue cells and can be oxidized to oxidized low-density lipoprotein. Moreover, oxidation of low-density lipoprotein easily causes atherosclerosis. Anthocyanin intake could reduce the level of LDL. LDL cholesterol can deposit in the arterial wall of the blood vessels such as the heart and brain, gradually forming atherosclerotic plaque, blocking the corresponding blood vessels, and finally causing coronary heart disease and stroke. And the LDL cholesterol can be decreased by anthocyanins. Meanwhile, anthocyanin-rich blueberry increases high-density lipoprotein (HDL) cholesterol, thereby preventing the risk of atherosclerosis because HDL can promote the reverse transport of cholesterol and clear cholesterol in peripheral tissues. Anthocyanins could decrease triacylglycerol (TAG) and TAG/HDL cholesterol ratio. Additionally, anthocyanins decreased the serum high-sensitivity C-reactive protein (hsCRP) levels, soluble vascular cell adhesion molecule-1 (sVCAM-1), and plasma interleukin (IL)-1β levels significantly in people with hypercholesterolemia (Zhu et al. 2013).

As previously mentioned, oxidation of low-density lipoproteins easily causes atherosclerosis, suggesting that oxidation reaction plays an important role in atherosclerosis. Malondialdehyde is the end product of lipid peroxidation, which can

exacerbate membrane damage. 8-OHdG is a biomarker of DNA oxidative damage. Anthocyanin supplementation can improve the ferric-reducing ability of plasma (FRAP), total antioxidant capacity (TAC), and oxygen radical absorbance capacity (ORAC) and reduce the production of malondialdehyde, 8-OHdG, and isoprostane. Abnormal glucose metabolism could increase the risk of CVD. Anthocyanins could reduce body mass index (BMI), fasting blood glucose, 2-hour postprandial blood glucose, and glycated hemoglobin. Anthocyanin-rich cranberry juice could reduce serum glucose, thereby decreasing apolipoprotein-B (apo-B) and increasing apolipoprotein-A1 (apoA-1) and paraoxonase-1 (PON-1) activity in type 2 diabetic male patients and decreasing the risk of CVD. Furthermore, anthocyanin intake was inversely proportional to the incidence of hypertension. Blueberry consumption could decrease central systolic blood pressure (cSBP), central diastolic blood pressure, mean arterial pressure (MAP), augmentation index, pulse wave velocity (PWV), brachial-ankle pulse wave velocities, and intima-media thickness, and the increased production of nitric oxide decreases blood pressure and arterial stiffness. Moreover, anthocyanin supplementation could decrease spontaneous and oxidative hemolysis and the number of activated platelets, thereby ameliorating CVD risk.

12.6.2 Protective Effects of Anthocyanin on Type 2 Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic disorders caused by defects in insulin secretion and function and characterized by long-term hyperglycemia, accompanied by a series of complications such as diabetic nephropathy, ischemic heart disease, and stroke. As a result, DM and its comorbidities cause huge healthcare burden and high mortality worldwide. Nearly 415 million people had type 2 diabetes mellitus (T2DM) in 2015, and it is expected to rise to 642 million by 2040 (Xu et al. 2018). So we must develop effective interventions and policy recommendations to alleviate these pressing public health issues. The causes of DM include genetic and environmental factors and obesity, resulting from eating too much and engaging in less physical activity, which is the major environmental factor for T2DM. That is, lifestyle and dietary habits are the major factors that determine the development and progression of obesity and T2DM. And T2DM is mainly caused by insufficient insulin secretion and insulin resistance. A lot of evidence indicates that dietary modifications such as fruit and vegetable intake could prevent these chronic degenerative diseases for the presence of many bioactive compounds. Among these bioactive compounds, anthocyanins especially attracted much attention for its wide distribution in plant foods and potent bioactivities. Wedick's team found that higher consumption of anthocyanins has a lower risk of T2DM, and higher intake of anthocyanin-rich foods such as blueberries, apples, and pears has also a lower risk of T2DM.

A meta-analysis of 8 prospective studies of a total of 312,015 participants suggests that anthocyanin intake was inversely associated with T2DM risk (Xu et al. 2018). Basal glycemia and postprandial blood glucose are two important indicators for diabetes diagnosis. Among them, basal glycemia can reflect the

function of islet β-cells and generally represent the secretory function of basal insulin. Postprandial blood glucose represents the blood glucose level after glucose load, and it is the earliest clinical manifestation before the onset of T2DM. Anthocyanin extracts could reduce the augment of postprandial glycemia and basal glycemia in T2DM patients. Insulin is a protein hormone secreted by pancreatic islet β-cells in the pancreas by endogenous or exogenous substances such as glucose, lactose, glucagon, etc., and insulin is the only hormone in the body that lowers blood sugar, and hyperinsulinemia is often a compensatory manifestation of insulin resistance. Anthocyanins could reduce the augment of insulin and dose dependently decrease insulinemia in prediabetic individuals. Insulin resistance refers to the decrease in the efficiency and utilization of insulin-promoting glucose uptake, and the body's compensatory secretion of excess insulin produces hyperinsulinemia to maintain blood sugar stability. Anthocyanin can decrease peripheral insulin resistance due to the decrease of insulin concentration in blood. Insulin sensitivity is the degree of insulin resistance, and most people with type 2 diabetes have reduced insulin sensitivity. Anthocyanins could lower postprandial insulin peaks and improve insulin sensitivity in insulin-resistant patients, and it has the potential to mitigate adiposity in overweight men. Glycated hemoglobin (HbAlc) is a product formed by non-enzymatic glycation of hemoglobin and albumin in red blood cells in the case of hyperglycemia. And HbAlc is an indicator of overall glycemic control in diabetic patients. Anthocyanins could decrease HbAlc serum levels. Hippuric acid is the main nonprotein nitrogen metabolite in urine, and its content reflects the metabolism of the kidney, and the content of hippuric acid in diabetic patients is low. Anthocyanin supplement can increase fasting serum hippuric acid. In brief, the epidemiological research and clinical trials provide tangible proof that increased anthocyanin intake is correlated with a lower incidence of T2DM and suggest that overweight or obese individuals should consider consuming more anthocyanin-rich foods to prevent development and progression of T2DM.

12.6.3 The Neuroprotection Function of Anthocyanin

Neurosis is a group of mental disorders that are mainly manifested as anxiety, depression, fear, coercion, suspected symptoms, or neurasthenia with a very high incidence. The symptoms of neurosis are complex and diverse, and the typical experience is a psychological activity that the patient feels uncontrollable and thinks should be controlled. The neurosis patients are deeply painful and impede mental or social function, but without any verifiable organic sexual pathology basis. Some epidemiologic study shows that anthocyanins can alleviate several neurosis-related diseases such as depression, anxiety, and cognitive function. Depression is one of the most common neuropsychiatric disorders. Epidemiological study of 1572 Italian individuals reveals that anthocyanins are inversely correlated with depression symptoms (Godos et al. 2018). Anxiety disorder is the most common type of neurosis, with anxiety as the main feature. Anthocyanins could improve alertness and decrease

fatigue and have an anxiolytic effect. Furthermore, anthocyanin-rich food consumption could improve cognitive ability, including verbal learning and memory in individuals with cognitive impairment. And anthocyanins could improve immediate spatial memory and driving performance.

Monoamine oxidase (MAO) is a mitochondrial enzyme containing flavin, embedded in neurons and non-neuronal cells, which catalyzes the oxidation of monoamines in a variety of tissues, including the brain. For a long time, elevated MAO activity has been associated with the causes of depression, anxiety, and neurodegenerative diseases. Anthocyanin extracts can improve mood effectively by inhibiting monoamine oxidase-B in healthy young adults (Watson et al. 2015). Insulin-like growth factor-1 (IGF-1) is a neurotrophic factor that plays an important role in neuronal survival and brain function. IGF-1 function is impaired in Parkinson's disease. Cyclic glycine-proline (cGP) is a metabolite of IGF-1 and has neuroprotective effects by improving IGF-1 function. Measuring the level of cGP can better represent the bioavailability of IGF-1. Blackcurrant anthocyanins can ameliorate anxiety and depression by increasing the concentration of cGP to improve the function of IGF-1 in Parkinson's patients. In a summary, anthocyanin supplements can exert neuroprotection function in human through many aspects.

12.6.4 The Protective Effects of Anthocyanins on Visual Functions

Visual function is the ability of the human visual system to perform certain visual tasks. It mainly includes the ability to detect the presence of objects, distinguish the details of an object, perceive the color of an object, and distinguish visual objects from a visual background. However, the visual function is degraded due to improper use of the eye or excessive use of the eye in daily life. Recent human studies have shown that the intake of anthocyanins can improve visual function. Recent increases in the daily workload of video display terminals (VDTs) have led to an increase in various symptoms associated with eye fatigue, including eye pain, dry eye or excessive tearing, and blurred vision, which may impair vision health and vision quality. Many clinical studies have shown a significant association between eye fatigue and psychophysiological eye function, which can be assessed by objective ophthalmic parameters such as critical flicker fusion (CFF). Anthocyanin supplementation could alleviate the reduction of CFF and mitigate the symptoms of eye fatigue, VDT load-induced ocular fatigue sensation, ocular pain, eye heaviness, uncomfortable sensation, and foreign body sensation in VDT workers. Anthocyanin-rich fruits could decrease the dark-adaptation threshold and hasten the recovery of visual acuity after photobleaching. Glaucoma optic neuropathy is considered to be one of the leading causes of irreversible blindness worldwide, and insufficient blood supply to the retina and optic nerve is one of the primary risk factors. Anthocyanin extracts of blackcurrant could increase blood flows at both neuroretinal rim of the optic nerve head and peripapillary retina in normal tension glaucoma (NTG) patients (Ohguro et al. 2008).

12.6.5 Other Properties of Anthocyanins

Anthocyanins not only protect humans from suffering from CVD, T2DM, nerve-related diseases, and visual function degradation but also protect from colitis, liver diseases, cancers, and other diseases. Anthocyanin treatment could reduce fecal calprotectin levels and decrease endoscopic Mayo score and histologic Riley index on ulcerative colitis. Furthermore, clinical trial suggests that anthocyanin-rich bilberry extract can mitigate ulcerative colitis by decreasing the proinflammatory cytokines IFN-γ, tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), and the level of phosphorylated p65-NF-κB and enhanced the levels of Th17-cell-specific cytokine IL-22 and immunoregulatory cytokine IL-10 (Roth et al. 2016). Moreover, anthocyanins could decrease serum levels of hepatic biomarkers including serum γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels in healthy adult men with borderline hepatitis. Anthocyanins could significantly reduce plasma alanine aminotransferase, cytokeratin-18, M30 fragment, and myeloperoxidase in nonalcoholic fatty liver disease. Anthocyanin intake was inversely associated with the risk of esophageal cancer and colorectal cancer. These promising intervention results deserve further long-term clinical trials to evaluate the beneficial effects of anthocyanin consumption on colitis, liver diseases, and cancer. In general, anthocyanin supplement protects a wide variety of human diseases.

12.7 Anthocyanins and Disease Prevention

Disease burden is measured by financial cost, mortality, morbidity, or other indicators. To reduce the cost of the society, disease prevention is more meaningful than clinical therapy. Dietary polyphenols exert positive effects in promoting health and preventing a wide range of chronic diseases. According to abundant studies on animals and humans, anthocyanins are a group of splendid polyphenols that have been associated with disease prevention, including cardiovascular diseases, diabetes, neurodegenerative conditions, cancer, and so on. Anthocyanins possess antioxidant, antidiabetic, antihyperlipidemic, anti-inflammatory, anticarcinogenic, neuroprotective, chemotherapeutic, and antiulcer activities.

12.7.1 Anthocyanins Prevent Metabolic Syndrome-Related Diseases

Metabolic syndrome (MetS) is represented by a group of interrelated metabolic disorders, which is accompanied by overweight or central (intra-abdominal) obesity, insulin resistance, cardiovascular diseases, and hepatic steatosis. MetS now affects 30–40% of adults, especially at a high rate in western countries. Anthocyanin-rich foods were reported to reduce the risks of metabolic syndrome-related diseases.

Obesity is presented as excess fat accumulation due to the unbalanced energy uptake and consumption. Commonly obesity is not considered seriously by individuals because of the high frequency and the impalpable injury. However, it is the basis of various metabolic syndrome-related diseases. Anthocyanin extracts have shown to prevent weight gain in mice, which is mainly on the white adipose tissue. Anthocyanin supplement significantly reduced body weight gain and suppressed fat accumulation in mesenteric adipose tissue in a high-fat diet mice model, reduced retroperitoneal adipose tissue, and decreased the adipocyte size of the epididymal fat (Jiang et al. 2018a). Meanwhile, anthocyanin treatment reduced the plasma content of free fatty acids, triglycerides, and cholesterol in obese mice. Chronic, low-grade inflammation is closely associated with obesity, which is also the trigger of many other chronic diseases. Anthocyanins decreased the level of F4/80 and downregulated the expression of TNF-α, IL-6, and nuclear factor kappa B (NF-κB). Adipose tissue is a consequence of excess lipid accumulation but also a cause of metabolic disorders. Adipokines are adipocyte-derived secretory factors which mediate many signaling cascades in target tissues, including insulin sensitivity and other procedures of energy homeostasis. Adipokines such as leptin and resistin are secreted from fat tissues, which mediate energy metabolism and insulin resistance. Serum adiponectin increased and the serum insulin and leptin level deceased by the treatment of anthocyanins, which indicated the improvement of adipokines. Interestingly, despite the abundant sugar, supplementation of the anthocyanin-rich fruit or the fruit juice, they have shown no promotion of body weight, on the contrary reduced the lipid accumulation (Wu et al. 2013).

Anthocyanins reduce the fasting glucose, total cholesterol, triglyceride levels, LDL, and glucose tolerance. Anthocyanins prevent the progression of type 2 diabetes by upregulating glucose transporter 4 (GLUT4) proteins' translocation in the membranes of heart and skeletal muscle tissues, which additionally promote energy usage. Anthocyanins could increase the activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ) in adipose tissue and skeletal muscles, thereby increasing secretion of adiponectin and leptin. Anthocyanins can also activate AMP-activated protein kinase (AMPK) and downregulate retinol-binding protein 4 (RBP4) expression to mediate glucose metabolism. In addition, anthocyanins reduce the apoptosis of pancreatic cells and protect the function of secreting insulin. Anthocyanins also alleviate insulin resistance, upregulate insulin receptor, and sensitize the insulin signaling. Anthocyanins could trap methylglyoxal (MGO) and reduce the carbonyl stress and the formation of advanced glycation end products (AGEs), which is a risk factor of diabetes (Chen et al. 2014).

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in the world. Anthocyanins could reduce the high production of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the reactive oxygen species (ROS), which indicated less hepatocyte damage. As previously mentioned, anthocyanins could reduce the level of plasma TG, LDL, and glucose, which are also the risk factors of NAFLD. Additionally, it can reduce the level of free fatty acid and

suppress the level of inflammatory cytokines in blood circulation. In the liver, anthocyanins inhibit lipid droplet accumulation in hepatocytes and ameliorate liver steatosis. Anthocyanins could reduce the lipid synthesis by mediating the protein expression of acetyl coenzyme A carboxylase (ACC) activities, sterol regulatory element-binding protein-1 (SREBP-1), fatty acid synthase (FAS), and glycerol-3-phosphate acyltransferase (GPAT). Furthermore, anthocyanins protect the integrity of mitochondrial and promote the lipid oxidation enzyme PPARα, CPT1 protein and gene expression of acylCoA-oxidase.

Accompanied by the injury of hepatocytes, inflammatory cells are infiltrated into the liver tissue from the blood circulation, the resident macrophage Kupffer cells are activated, and inflammatory cytokines as well as chemokines were generated, which will aggravate cell damage and the activation hepatic stellate cells. Supplementation of anthocyanins significantly inhibited inflammatory cell infiltration and secretion of cytokines, which prevented progression of nonalcoholic steatohepatitis (NASH) and liver fibrosis. Anthocyanin-rich extract can enhance the level of PI3K and p-Akt in the liver. Anthocyanins can also target AMPK phosphorylation and autophagy, thus suppressing the activation of hepatic stellate cells.

Heart attack and fibrosis could be consequences of diabetes mellitus. Anthocyanin treatment significantly reduced cardiac hypertrophy and fibrosis and protected the heart. Endothelial cells also produce a wide range of factors that mediate cellular adhesion, smooth muscle cell proliferation, and vessel wall inflammation. Moreover, they act as a semipermeable barrier that controls blood-tissue exchange of fluids, nutrients, and metabolic wastes from the intravascular compartment to the interstitium. Anthocyanins alleviate endothelial dysfunction and apoptosis and attenuate oxidative stress. Anthocyanins could target the phosphorylation levels of AMPK, promote the level of eNOS and the generation of NO, and elevate cyclic GMP (cGMP) in the aorta. Moreover, they can also suppress iNOS level and restrain oxidative damage. Anthocyanins promoted the expression of ATP-binding cassette transporter G1 (ABCG1) and lower cholesterol, mainly 7-ketocholesterol (7-KC). Anthocyanins could also inhibit inflammatory cytokine secretion from endothelial cells. Protecting of endothelium of anthocyanins lead to a much less aortic lesion, and a better function of relax or constrict the vessel. Additionally, compared to aspirin, anthocyanins have less side effects and could be a potential antiplatelet agent that prevents atherosclerosis and other cardiovascular diseases.

12.7.2 Anthocyanins Prevent Neuro-damage

Insulin resistance is related to hyperphosphorylation of microtubule-associated protein tau in the hippocampus, which may cause the development of Alzheimer's disease. Anthocyanin-rich extract reduced the level of inflammation and oxidative

stress, improved insulin sensitivity and learning/memory performance, and prevented tau phosphorylation in the hippocampus. Anthocyanins might regulate FKBP52 (FK506-binding protein 52) and prevent Alzheimer's disease. FKBP52 has been found to inhibit tau protein aggregation. The docking score of anthocyanins for FKBP52 is greater than for FK506 for the FK1 domain. Anthocyanins cause FKBP52 structural variations to be the same as FK506 when the protein and ligand interact. Anthocyanins as legends will make PKBP52 stable, and different anthocyaninshavevariousbindingresults:C3G>Pt3G>Pa3G>D3G>FK506>P3G>M3G (Hung et al. 2014). Anthocyanins could improve hippocampal insulin signaling in amyloid beta-infused rats, thus preventing cognitive dysfunction. In addition, anthocyanin treatment reduced the generation of amyloid protein. Protocatechuic acid as a metabolite of anthocyanins can also attenuate brain mitochondrial dysfunction, thereby preventing oxidative stress.

Vascular dementia is one of the challenging problems of elderly. Oral treatment of mulberry extract for rats suffering from vascular dementia showed enhanced memory and increased densities of the neuron but less apoptosis and oxidative stress. The neuroprotective effect of the extract might also be associated with the increased cholinergic function. LPS has been reported to affect neuronal cells via activation of microglia as well as to directly initiate neuroinflammation. Anthocyanin treatment at a dose of 24 mg/kg/day for 2 weeks prevented ROS production, inhibited neuroinflammation and neurodegeneration, and improved memory functions in LPS-treated mice. Anthocyanins reversed the activation of JNK, prevented neuroinflammation by lowering the levels of inflammatory markers (p-NF-κB, TNF-α, and IL-1β), and reduced neuronal apoptosis by reducing the expression of Bax, cytochrome c, cleaved caspase-3, and cleaved PARP-1 while increasing the level of survival proteins p-Akt, p-GSK3beta, and anti-apoptotic Bcl-2. Anthocyanin treatment increased the levels of memory-related pre- and post-synaptic proteins and improved the hippocampus-dependent memory in the LPS-treated mice. Anthocyanins reduced the level of phospho-c-Jun N-terminal Kinase 1 (p-JNK). Anthocyanin treatment also reduced activated astrocytes and microglia in the cortex of LPS-injected mice, as indicated by reductions in GFAP and Iba-1, respectively.

Parkinson's disease (PD), one of the most common neurodegenerative disorders, is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) to the striatum (ST) and involves oxidative stress. In cell model, SH-SY5Y cells stressed with 6-hydroxydopamine (6-OHDA), mulberry extract (ME) downregulated ROS and NO generation; mediated Bcl-2 and Bax proteins, mitochondrial membrane depolarization, and caspase-3 activation; and significantly protected the cells from neurotoxicity in a dose-dependent manner. In mesencephalic primary cells stressed with 6-OHDA or 1-methy1-4-phenylpyridinium (MPP^+), pretreatment with ME also protected the dopamine neurons. In the subacute mouse PD model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), ME showed a preventative effect against PD-like symptoms (bradykinesia) in the behavioral test and prevented MPTP-induced dopaminergic neuronal damage in an immunocytochemical analysis of the SNpc and ST (Kim et al. 2010).

12.7.3 Anthocyanins Prevent Retinal Damage

Retinal damage is becoming a more and more severe challenge to mankind in all ages. Anthocyanins are reported to inhibit retinal cell apoptosis and photochemical damage in rats. Anthocyanins inhibited the expression of AP-1, upregulated NF-κB and P-IκB, and decreased the level of caspase-1. In human studies, bilberry extract treatment on the eye fatigue induced by acute video display terminal had some favorable effects. Bilberry supplementation induced a reduction in critical flicker fusion (CFF), but the near point accommodation (NPA) was not changed. Ocular fatigue sensation, ocular pain, eye heaviness, uncomfortable sensation, and foreign body sensation were mitigated more in the bilberry group. Thus supplementation improved some of the objective and subjective parameters of eye fatigue induced by VDT loads.

Cataracts have become the leading cause of blindness which is mediated by oxidative stress. Anthocyanins prevented oxidative stress and reduced photoreceptor cells. Anthocyanins with an *N*-trimethyl chitosan (TMC)-coated liposomes transport to a depth of 40 μm in the cornea, where SOD, catalase in lens elevated, and lipid peroxidation prevented.

12.7.4 Anthocyanins Prevent Cancer

The antitumor potential of anthocyanins is reported based on a wide variety of biological activities. However, the more meaningful aspect is the anti-tumorigenesis effect, due to the antioxidant, anti-inflammation, and anti-mutagenesis activities inhibiting proliferation, invasion, angiogenesis, and metastasis.

Diets containing 5% of either black or red raspberries, strawberries, blueberries, noni, and a double dagger ai or wolfberry were used to treat *N*-nitrosomethylbenzylamine-esophagus cancer model. All berry types were about equally effective in inhibiting tumorigenesis in the rat esophagus. They also reduced the levels of the serum cytokines, IL-5 and GRO/KC, and the rat homologue for human IL-8, and this was associated with increased serum antioxidant capacity. Lyophilized black raspberries (BRB) reduced the incidence of esophageal cancer from 100% in model group to 81.5% in the rats treated with BRB ($p < 0.05$). The tumor multiplicity was reduced from 4.73 +/− 0.45 tumors per esophagus in the model group to 1.44 +/− 0.26 in rats treated with intervention with BRB. Using bioassay-directed fractionation, the anthocyanins in BRB were found to be the most active constituents for downregulation of carcinogen-induced NF-κB and AP-1 expression in mouse epidermal cells in vitro. An important study has shown that anthocyanins are the main bioactive component in BRB to prevent cancer growth. Animals were treated for 30 weeks to induce esophageal tumors; anthocyanin treatments (5% whole BRB powder, an anthocyanin-rich fraction, an organic solvent-soluble extract, each contained similar to 3.8 μmol anthocyanins/g diet) were about equally effective in reducing tumorigenesis in the esophagus, indicating that the anthocyanins in BRB have chemopreventive potential. The organic-insoluble (residue)

fraction was also effective, suggesting that components other than berry anthocyanins may be chemopreventive. The hexane and sugar diets were inactive (Wang et al. 2009).

Breast cancer is related to altering of estrogen metabolism. In an animal model, blueberry anthocyanins and black raspberry anthocyanins inhibited 17 beta-estradiol-induced mammary tumor incidence, volume, and multiplicity. In the mammary tissue, anthocyanin extracts inhibited the increase of cytochrome P4501a1 and 17β-HSD7 at different time points (6 weeks, 18 weeks, 24 weeks). In another study, the experimental diet rich in anthocyanins significantly delayed the first tumor appearance by 21 days; reduced estrogen-associated growth of pituitary prolactinomas, circulating prolactin, and estradiol levels; and offset estrogen-associated increases in mammary cell proliferation, estrogen receptor-alpha (ER-alpha), and cyclinD1. miRNAs related to the tumorigenesis that were either overexpressed (miR-182 and miR-375) or underexpressed (miR-127 and miR-206) following estrogen treatment were significantly protected by anthocyanin-rich diet (Aqil et al. 2016).

In an azoxymethane (AOM)-/dextran sodium sulfate (DSS)-induced mice colon cancer model, anthocyanin treatment led to a reduction of carcinogenesis in the colon and lower tumor multiplicity. In the mice model, supplement of purple potato extract reduced the injury caused by azoxymethane in aberrant crypt foci of colons. A less proliferating cell nuclear antigen (PCNA) and a greater apoptotic caspase-3 expression were found in the colon mucosal epithelial cells. Importantly, anthocyanin treatment maintained the balance of gut microbiota by decreasing the pathogenic bacteria and increasing the probiotics. Anthocyanins also caused demethylation of the SFRP2 gene promoter, resulting in increased expression of SFRP2, both at the mRNA and protein levels. Furthermore, the expression levels of DNMT31 and DNMT3B, as well as of p-STAT3, were downregulated by anthocyanins in these animals (Tian et al. 2018). In vitro, anthocyanin-rich extracts from purple potato suppressed human colonic SW480 cancer cell growth by arresting the cell cycle at G1 phase but not cytotoxicity. Anthocyanins can modulate multiple signaling pathways such as NF-κB, Wnt/-catenin, PI3K/AKT/PKB/mTOR, and ERK/MAPK in colon cancer.

It is important to reduce the side effects of chemotherapy. Phytochemicals including anthocyanins are also applied in clinical trials for the protection of the heart and reduction of cardiovascular diseases or cardiotoxicity induced by anticancer drug. Cisplatin can induce hepatotoxicity and oxidative stress. Anthocyanin-rich extract caused a marked reduction in the activities of alanine aminotransferase and aspartate aminotransferase after cisplatin administration.

12.7.5 Anthocyanins Prevent Other Chronic Diseases

Anthocyanins have been proved to prevent some other chronic diseases. Due to the antioxidant and anti-inflammatory ability of anthocyanins, they can suppress

the development of autoimmune arthritis. In a collagen-induced arthritis mice model, black soybean coat extract (mainly C3G) reduced the incidence of arthritis, according to the histological inflammation analysis, cartilage scores, and oxidative stress. Proinflammatory cytokines in the joints were suppressed by anthocyanins, and NF-κB was suppressed. Importantly, Th17 cell number in the spleen was decreased; the differentiation of Th17 from splenocytes and human PBMCs and Th17-associated genes in vitro was suppressed by the treatment of anthocyanins. Motor function is inversely correlated to the age. Osteoclast formation is a crucial action for the loss of bone in animals, especially accompanied by the decreasing of estrogen. Blueberry anthocyanins were proved to reduce bone loss in osteoporosis mice model and suppress osteoclast formation in vitro. Among them, delphinidin is the most efficient anthocyanidins to suppress microphage differentiation to osteoclast and suppressed the activation of NF-κB, c-fos, and Nfatc1 and master transcriptional factors. PCA as a metabolite of anthocyanins also inhibited LPS-induced bone loss in vivo, suppressed RANKL-stimulated expression of c-Fos and nuclear factor of activated T cells c1, and downregulated the level of p-JNK and osteoclastogenesis-related genes including 3-integrin, DC-STAMP, OC-STAMP, Atp6v0d2, CTR, and CtsK. Muscle atrophy is common in elderly. Delphinidin suppressed muscle weight loss through downregulating oxidative stress-related gene expression including Cbl-b, inhibited MuRF1 expression, and promoted miR-23a AND NFATc3 expression.

Anthocyanins are also beneficial for male reproductive ability. Cyanidin-3-*O*-*β*-D-glucopyranoside promoted erectile function in diabetes mellitus rat model. Anthocyanin minimized corporal apoptosis, increased the expression of endothelial eNOS and nNOS, and reduced the level of 8-OHdG. In an andropause animal model with low androgen, anthocyanin treatment increased the activity of SOD in serum and reduced oxidative stress. Anthocyanins reduced the level of apoptotic cells in the prostate. Oxidative stress in the testis is a threat of spermatogenesis. Under hypobaric hypoxia, blueberry extract reduced lipid peroxidation, decreased apoptosis, and increased GR and SOD activities in rat testis. In a chloropropanol-induced injury on male rat reproductive system, cyanidin-3-*O*-*β*-glucoside (C3G) improved the number and motility of the sperms, alleviating seminiferous tubule injury. Interestingly, C3G showed no influence on sexual hormone but increased the androgen receptor expression. Meanwhile, C3G reduced the oxidative stress and number of apoptotic cells and promoted the integrity of the blood-testis barrier in the testis. Additionally, C3G mediated the activation of p-ERK, p-JNK, and p53, which are related to the protection of Sertoli cells and spermatogenesis (Jiang et al. 2018b).

Anthocyanins have shown some potential on the protection of skin damage. In an acute skin photodamage mice model, Nano-C3G could effectively reduce the UVB-induced lipid peroxidation, malondialdehyde, and 8-hydroxy-2′-deoxyguanosine contents; downregulate p53, Bcl-2-associated X (Bax), and caspase-3 and caspase-9 expression; and balance the B-cell lymphoma-2/leukemia-2 ratio. Moreover, Nano-C3G (125, 250, 500 μM) improved the visual appearance, skin moisture, histologic appearance, and apoptotic index (based on

TUNEL staining) under UVB exposure (Liu et al. 2018). In a cell study, when HaCaT cells were exposed to UV radiation in the presence of nanoberries, their viability was maintained. In dermal fibroblasts, treatment with anthocyanins significantly reduced radiation-induced apoptosis and intracellular reactive oxygen species generation at 48 h. Furthermore, high dose of anthocyanins markedly decreased Smad3 mRNA expression and increased Smad7 mRNA expression post-irradiation. In mice, anthocyanin treatment significantly reduced the level of skin injury, epidermal thickness, and collagen deposition after irradiation. Treatment with anthocyanins significantly decreased the number of α-SMA, TGF-β, and Smad-3-positive cells after irradiation.

12.8 The Safety of Anthocyanins

Anthocyanin daily intake is larger; in addition to giving rich colors to nature, anthocyanins contained in plant foods also have a variety of physiological health and disease prevention effects, so they can be used as food additives and medical foods. Then, what is the cytotoxicity of anthocyanins? And what dose of anthocyanin is harmful to cells? In animal experiments, how many doses can cause harm to animals? How many anthocyanins are allowed to be taken in a daily diet, and what is the recommended daily meal amount? This is waiting for us to discuss.

12.8.1 Toxicity of Anthocyanin In Vitro

12.8.1.1 Cytotoxicity and Common Detection Methods

Cytotoxicity is a simple cell-killing event caused by cells or chemicals and does not depend on the cell death mechanism of apoptosis or necrosis. The correlation coefficients commonly used in cytotoxicity experiments are GI_{50} and IC_{50}.The values means concentration that inhibited 50% of the net cell growth.

12.8.1.2 Effects of Different Sources of Anthocyanins on the Activities of Different Cell Lines

The effects induced by the anthocyanin extract on the survival of different cell lines were analyzed in both dose-response and time-course experiments. Compared with cancer cells, anthocyanins have a greater concentration of effects on normal cell viability. The activity of PRSE (polyphenol-rich strawberry extract) was investigated in normal fibroblast cell lines of both murine (NIH-3 T3) and human (WI38) origin, and they are significantly less sensitive to PRSE with respect to cancer cell lines (mean IC_{50} of 3.24 ± 0.14 mg/mL and 1.68 ± 0.77 mg/mL) (Amatori et al. 2016). Lili et al. have studied and evaluated the cytotoxic effects of three different berry extracts on RAW264.7 cells (macrophages) at three different concentrations (100, 150, and 200 mg/mL). The data from MTS assay showed no significant changes in

cell viability, indicating that three berry extracts were not cytotoxic at dosage up to 200 mg/mL (Li et al. 2013). Other research also shows the growth of normal cells is less significantly inhibited by anthocyanin extracts than that of cancer cells. The IC_{50} values of various normal cells are more than twice that of corresponding cancer cells, reflecting the advantage of small side effects of natural products. About cancer cells, the treatment of cells with purified anthocyanin extract or crude extracts from *Hibiscus sabdariffa* calyx at 5–800 μg/mL did not show significant cytotoxic effects on Caco-2, HepG-2, HCT8, and A549 cell lines (Maciel et al. 2018). The *C. cyanus* petals aqueous extract exhibited IC_{50} and GI_{50} (> 900 μg/mL) values for HepG-2, Caco-2, and A549 cell lines, meaning low cytotoxicity. Based on the stress oxidative assay, the extract exhibited prooxidant action (10–100 μg/mL) but did not cause damage or cell death (Escher et al. 2018). Similar results were also described by Amatori et al. for A17 (breast cancer cell line) cells which show higher response to PRSE exposure (IC_{50} of 1.14 ± 0.29 mg/mL). At this stage, there have been few studies on the toxicity test of cells with certain anthocyanins, and more attention has been paid to the protective effect of anthocyanins on cell damage caused by certain harmful elements.

12.8.2 Anthocyanin in Animals

12.8.2.1 Food Safety Toxicology Evaluation

Anthocyanin pigments are required as a natural food colorant to be evaluated for their safety. Food safety toxicology evaluation has four stages:

First stage: acute toxicity test, LD_{50}, combined with acute toxicity, maximum tolerated dose method
Second stage: genotoxicity test, traditional teratogenic test, and 30d feeding test
Third stage: subchronic toxicity test-90d feeding experiment, reproduction test, and metabolic test
Fourth stage: chronic toxicity test (including carcinogenic test)

The table presented some common sources of anthocyanin toxicology safety data (Table 4).

Among them, grape skin extract-grape skin anthocyanin can be safely used in food and is a coloring agent according to GRAS (Generally Recognized as Safe). In addition to being used as a coloring agent, anthocyanins extracted from blueberries can also be used as an antioxidant nutrient supplement. The products on the market are tablet products, each containing 50 mg of anthocyanin. In general, common anthocyanins from different substances have different effects on animal toxicity tests. The daily intake of anthocyanins in the US diet is estimated to be between 180 and 215 mg (blueberry anthocyanin). Therefore, the intake of most anthocyanins between these measurements will not cause harm to the human body.

Table 4 Toxicological tests of anthocyanins from different sources

Anthocyanin source	LD_{50}	Micronucleus test	Sperm deformity test	Toxicity grading standard	Remarks
Purple corn	>21.50 g/(kg·bw) rat oral	–	No teratogenic effect	Safe	
Grape skin	15 g/(kg·bw)(male rat oral) 10.8 g/(kg·bw) (female mice oral)	–	No teratogenic effect	Safe	ADI:0–2.5 mg/kg(JECFA2006; ADI value first mentioned in 1982
Black bean	19 g/(kg·bw)(mice oral)	No teratogenic effect			
Blackcurrant	10 g/(kg·bw) (rat, mice oral)	–	–		In the USA, the recommended intake is 180–215 mg/day
Roselle	9260 mg/(kg·bw) (mice oral)				
Myrica		–	No teratogenic effect		
Cowberry	>25 g/(kg bw) (mice oral)	–	No teratogenic effect		Weak accumulation

Note: negative; ADI, daily allowable intake of people

12.8.3 Population Investigation

Cell and animal experiments by anthocyanins indicate that anthocyanins are safe in a certain concentration range and have certain anti-inflammatory, anti-oxidation, and anticancer effects. Based on the species differences between experimental animals and humans, scientists have been working in recent years to extrapolate research findings from experimental animals outside the body. In daily life, most of the dark fruits and vegetables we eat contain different amounts of anthocyanins. This benefit requires us to further discuss anthocyanins through human clinical trials. Wilhelmina's Crowd experiment, subjects daily intake 250 mL of wild blueberry juice (containing 216 mg (448 μmol cyanidin 3-glucoside equivalents)) for a period of 36 days. The results has shown that the excretion content of anthocyanins was basically unchanged within 7 days, and the anthocyanin content in the long-term body circulation is gradually reduced (Kalt et al. 2017).

The emotional and cognitive changes of young people after adding purple grape juice were studied (the content of anthocyanin in purple grape juice was 136.6 mg per 200 mL), which showed that people's cognition, memory, and concentration improved in a certain period of time (Haskellramsay et al. 2017). In one population, the utilization rate of different anthocyanins in the bilberry extract was studied in the intestine. The cranberry extract contained 15 different kinds of anthocyanins, and each person ingested 10 g of untreated onetime. About 1.3 ± 0.9 μmol of anthocyanins and 20.3 ± 5.2 μmol of degradation products were excreted via urine in healthy volunteers ($N = 5$) (Mueller et al. 2018; Mueller et al. 2017). Myoungsook conducted an experiment in which 32 people received 2.5 g of black bean extract every person per day (anthocyanin content, 12.58 mg/g; cyanidin-3-glucoside, 68.3%) for 8 weeks. The treatment has shown a strong improvement in plasma lipid profiles, which is related to the reduction of abdominal fat (Lee et al. 2016). Therefore, further confirmation of anthocyanins as food additives can be added by human studies.

To date, anthocyanin toxicity has not been found under ordinary dietary conditions in humans. In short, the anthocyanins ingested in the daily diet will not cause damage to the human body. According to the current anthocyanin population intervention test results, no adverse reactions will occur if the anthocyanin intake level reaches 320 mg/d.

12.9 Development and Utilization of Anthocyanins

Anthocyanins are a natural, environmentally friendly food source pigment, which are abundant and nontoxic and provided specific nutritional and health value and pharmacological effects. It has tremendous potential for application and development in food, functional foods, medical and health, cosmetics, chemical production, and so on. The extraction process is a key link in the production and use of anthocyanins. Currently, the main extraction methods of anthocyanins prevailingly include organic solvent extraction, water extraction, supercritical fluid extraction, ultrasonic-assisted extraction, microbial fermentation extraction, enzymatic extraction, microwave assisted extraction, liquid static high pressure-assisted extraction, subcritical water extraction, pressurized solvent extraction, dual aqueous phase extraction, and high-voltage pulsed electric field-assisted extraction.

Different purities of anthocyanin extracts can be applied to products in different fields. For example, anthocyanin crude extracts can be mainly used as food additives, raw materials for food, and functional foods and for medical purposes. The high purity monomer of anthocyanin is principally used as a standard in scientific research, due to its high cost. At present, the relatively mature fields of development and utilization concerning anthocyanin extracts are food additives, deep processing of anthocyanin-rich products, and dietary supplement or functional foods.

12.9.1 Pigments Used as Additives

As one of the indispensable additives in the food industry, pigments play an important role in improving the sensory quality. Pigments can be classified into artificial synthetic pigments and natural pigments according to their sources. Synthetic pigments include amaranth, carmine, sunset yellow and so on. Natural pigment is the term that refers to an edible pigment which is derived from plants, fruits, animals, and microorganisms, such as anthocyanins and carotenoids. Natural pigments from animal and plant tissues, are safe and nontoxic and have no side effects. Meanwhile, a myriad of pigment, which has biological activities and nutritional strengthening effects, can show attractive colors and provide a pleasant feeling by adding to foods. However, synthetic pigments are mostly from coal tar fuels. The chemical structures of synthetic pigments are azo compounds, which can form β-naphthylamine and α-amino-1-1 naphthol in the process of metabolism. Compared to natural pigments, synthetic pigments possess certain potential carcinogenicity, have a limited scope of application. They are popularly applied in soft drinks and confectioneries. Nevertheless, it is prohibited in meats, infant foods, and biscuits. The principal pathways of toxically synthetic pigments are (1) the toxicity of chemical substances injures human health; (2) the hazardous substance can be generated through metabolic process; and (3) it is contaminated by heavy metals such as arsenic, lead, or other toxic and harmful compounds in the process of synthesis. Therefore, the development and utilization of natural pigments is an inevitable trend. Furthermore, its application prospects are broad.

Anthocyanins are natural pigments derived from plant-based raw materials, which are vital substitutes for synthetic pigments, due to the good water solubility. It can be used as additives in the food industry. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) uses toxicological data to evaluate the safety of anthocyanins as food additives. Ultimately, it is determined that anthocyanins have "minimal toxicity" or are "practically nontoxic" and can be included in the natural pigment food additive (Table 5). Currently, anthocyanins are mainly used in $pH < 4.5$. Anthocyanin extracts have been approved by the Chinese Ministry of Health for use, such as for beverages, jams, candies, dairy, and ice cream (Table 6). Anthocyanin extracts as food additives are not need to be certified, which are utilized in beverages, dairy, cakes, and so on, according to FDA standard (Table 7). Anthocyanin extracts are widely used in European Union countries and in Japan. And EU has summarized the levels of usage of anthocyanins (E 163) as food additive in the five population groups (Table 8).

12.9.2 Deep Processing of Anthocyanin-Rich Products

Anthocyanins from purple cabbage, purple potato, blueberry, black rice peanuts, etc. going through deep processing have splendid development prospects.

Recently, anthocyanin-rich berry products have been widely developed and utilized and also are very popular and have a vast market at home and abroad. Anthocyanins

Table 5 General standard for food additives (grape skin extract as anthocyanin)

Food category	Max level (mg/kg)
Flavored fluid milk drinks	100
Cream analogues	150
Milk and cream powder analogues	150
Dairy-based desserts (e.g., pudding, fruit, or flavored yogurt)	200
Fat-based desserts excluding dairy-based dessert products of food category	200
Edible ices, including sherbet and sorbet	100
Canned or bottled (pasteurized) fruit	1500
Jams, jellies, marmalades	500
Fruit-based spreads (e.g., chutney) excluding products of food category	500
Fruit preparations, including pulp, purees, fruit toppings, and coconut milk	500
Fruit-based desserts, including fruit-flavored water-based desserts	500
Fermented fruit products	500
Fruit fillings for pastries	500
Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine, or soybean sauce	100
Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter)	100
Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables) other than food category	100
Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soybean products of food categories	100
Cocoa-based spreads, including fillings	200
Cocoa and chocolate products	200
Imitation chocolate, chocolate substitute products	200
Soft candy	1700
Chewing gum	500
Decorations (e.g., for fine bakery wares), toppings (non-fruit), and sweet sauces	500
Cereal and starch based desserts (e.g., rice pudding, tapioca pudding)	200
Crackers, excluding sweet crackers	200
Bread-type products, including bread stuffing and bread crumbs	200
Egg-based desserts (e.g., custard)	200
Mustards	200
Soups and broths	500
Emulsified sauces and dips (e.g., mayonnaise, salad dressing, onion dip)	300
Non-emulsified sauces (e.g., ketchup, cheese sauce, cream sauce, brown gravy)	300
Mixes for sauces and gravies	300
Dietetic foods intended for special medical purposes	250
Dietetic formulae for slimming purposes and weight reduction	250
Dietetic foods	250

(continued)

Table 5 (continued)

Food category	Max level (mg/kg)
Food supplements	500
Water-based flavored drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	300
Cider and perry	300
Wines (other than grape)	300
Distilled spirituous beverages containing more than 15% alcohol	300
Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low-alcoholic refreshers)	300
Snacks – potato, cereal, flour, or starch based (from roots and tubers, pulses, and legumes)	500
Processed nuts, including coated nuts and nut mixtures(with, e.g., dried fruit)	300

Table 6 Natural anthocyanin approved in China

Source	Recommended range	Maximum (g/kg)
Black bean red	Sweets, pastries, fruit and vegetable juice, flavor drinks, and assembled wine	0.8
Blackcurrant red	Cakes, soda drinks, and wine	According to production requirement
Red rice red	Modified milk, cold drinks, sweets, and wine	According to production requirement
Sweetberry honeysuckie red	Cold drinks, sweets, pastries, flavored drinks, and wine	3.0
Fall kwai red	Sweets, cakes, jelly, soda drinks	0.25
Roselle red	Sweets, fruit and vegetable juice, flavor drinks, and assembled wine	According to production requirement
Grape red skin	Cold drinks, jam, pastries, soda drinks, flavored drinks and assembled wine	1.0
Mulberry red	Fruit cakes, sweets, jellies, flavored drinks, and wine	5.0
Cowberry red	Cold drinks, fruit and vegetable juices, and flavor drinks	According to production requirement

are a vital series of phenolic compounds in grape and wine. They are prevailingly distributed in the vacuoles of 3–4 layers of cells of grapes. If whole grapes are adopted for fermentation, anthocyanins can infiltrate the fermentation broth during the process to obtain wine. According to statistics, the yield of wine in the global market reaches up to 24.67 billion L, and wine accounts for above 80%. The new wine contains abundant anthocyanins, predominantly anthocyanin 3-*O*-glucoside and delphinidin 3-*O*-glucoside. As researches put it, it is universally acknowledged that anthocyanins

Table 7 Source and the recommended range of anthocyanins approved by FDA in USA

Source	Recommended range
Ipomoea hederacea	Sweet wine
Brassica oleracea	Ice cream, yogurt, sweet milk
Hibiscus sabdariffa	soft drink, sweets
Vaccinium oxycoccos	Fruit pulp, sour and spicy pulp, jelly, fruit wine, solid drink, powder containing sour sweet
Clitorea tematea	Cocktail, solid drink premix, rice flour cake with sweet gum, or solid food
Grewia asiatica	Drink
Rubus idaeus	Fruit juice

Table 8 Summary of anticipated exposure to anthocyanins (E 163) from its use as food additive using maximum reported use levels in five population groups (mg/kg bw/day)

	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (>65 years)
Mean	1.5–4.0	1.5–4.7	1.0–2.5	0.7–1.9	0.5–1.1
High level	3.2–6.9	2.7–7.8	1.6–3.9	1.1–3.4	0.9–2.3

enjoy paramount effects on skin care and prevention of cardiovascular diseases. In addition, 7% grapes are mainly used for processing juices, jams, and other products, such as grape juice, grape enzymes, and grape jam.

Besides grapes, other anthocyanin-rich berry products have been widely exploited. Other related products of blueberry, cranberry, and others exert a tremendous fascination on consumers, for example, blueberry wine and cranberry wine. In addition to wine, the technology of berry jam development has matured. The sauce and jam of berry invariably manifest promising economic performance. Meanwhile, related products include grains, such as preserved vermicelli and beverage, which are made by purple sweet potato.

12.9.3 Dietary Supplement or Functional Foods

Expecting the basic effects on coloring, much of the research in anthocyanins at home and abroad has examined the favorable biological activities, namely, antioxidation, antiaging, anti-inflammatory, neuroprotective, and promote re-synthesis of erythrosin (Yang et al. 2009). Previous investigations of long-term use of anthocyanin-rich products had emphasized on maintaining vascular health and preventing cardiovascular disease. Anthocyanins are indicative of potential functions in reducing inflammation and protecting the arterial wall from damage. Simultaneously, it is more preferable than vitamins C and E in protecting arteries. Consequently,

Table 9 Anthocyanins used in functional food

Sources	Purity	Functions	States
Blueberry	80 mg/capsules	Improve vision	Norway
Cranberry powder, cowberry extractive, blueberry powder	2 g/100 g	Improve immunity	China
Bilberry powder	5.88 g/100 g	Relieve visual fatigue	China
Cowberry extractive	6.7 g/100 g	Relieve visual fatigue	China
Blueberry extractive	28.88 mg/capsules	Improve vision	USA
Cranberry extractive	18 mg/capsules	Improve immunity, protective urinary system	Germany
Blueberry extractive	12.5 mg/capsules	Anti-oxidation, improve vision	Canada
Bilberry extractive	20 mg/capsules	Relieve fatigue, improve vision	UK
Nordic strawberry extractive	90 mg/capsules	Relieve eye fatigue, improve vision	Japan

anthocyanins, which are called "pigment nutrients," can be extensively used as crucial functional foods and raw material in pharmaceutical.

Presently, there are a multitude of health products produced from anthocyanins worldwide which includes tablets and capsules (Table 9). The functions as principally claimed are relieving visual fatigue and strengthening immunity, anti-oxidation, and so on.

In a word, combining the advantages of superior physiological efficacies, extensive sources, no side-effects and with the support of advanced production technology, the development and utilization of anthocyanin-related products have feasibility in food industry and economy. However, many external factors like pH, metal ions, oxygen, and enzymes can seriously affect the stability of anthocyanins in the food processing and storage. Therefore, the instability exceedingly limits its development and utilization. It is of great significance to discuss the stability and expand its application by physical-chemical and biological means. The main methods include co-pigmentation, molecular modification, microencapsulation, complementary synergy, biotechnology, and so on. Generally, the modification of anthocyanins to make up for deficiency makes its application as natural pigments more extensive. Hence, it is a problem that cannot be ignored for the development and utilization of anthocyanin-rich products.

12.10 Perspective

Anthocyanins as a group of dietary polyphenols are widely applied in the food industry and could provide potential benefits for humans. We systematically introduced the source, chemical activity, bioactivity, and functions of anthocyanins, which show a universal source and a broad development potential.

Also, some of the unclear issues are still calling for further investigation. (1) Appropriate dosage of anthocyanins to be used – in animal studies or clinical studies – is still uncertain (Kim et al. 2013). The recommended anthocyanin intake has not been certainly determined yet, which limited the application of anthocyanins for daily consumption and product development. (2) The structural and functional relationship of anthocyanins is still not well evaluated. Structural issues are quite important for influencing the bioavailability and bioactivity of anthocyanins. Additionally, different anthocyanin-rich foods contain different prominent monomers, and the structural-functional study will impact the value of their development. (3) The direct molecular targets of anthocyanins in cells are still not well illustrated. Although some studies indicated that anthocyanins can directly bind to IL-17, NF-κB as a modulator, it is still a mystery of the molecular targets, as to anthocyanins showed so broad-spectrum effects on many signaling pathways. (4) The anthocyanin-related signal transduction pathways are still needed for further exploration. For instance, autophagy is crucial in adipocyte, hepatocyte, and hepatic stellate cell apoptosis or activation (Zhang et al. 2012); however, anthocyanins mediation on it is merely studied yet. (5) To illustrate the effects of anthocyanins in metabolic syndrome, the comparison studies about anthocyanins and some other well-known drugs or compounds are needed, which is a valuable source of anthocyanin product development. (6) Studies of anthocyanins on other animal models and cells are needed. With no doubt, all the animal or cell models have some limits. The hazard factor exposures in our daily life are much more complex. Thus the studies of disease prevention or therapy need different models to mimic the actual environment. (7) The stability, bioavailability, and effect of anthocyanins in vivo are still controversial problems. Some studies try to enhance the stability and to make nano-encapsulation of anthocyanins, which may have shown better bioavailability and sustain longer time than the unembedding anthocyanins (Bennet et al. 2014). However, some studies proved that its metabolites have better bioactivities. Whether it is better to improve or inhibit the degradation of anthocyanins is still unknown.

All in all, anthocyanins are important in our daily life, and it has a bright future for the application in food additive production and for disease prevention and therapy.

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Dietary Triterpenoids

13

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Abstract

Triterpenoids, as a more important and the largest group of phytochemicals, exist in thousands of medicinal plants from which more than 20,000 substances with up to 100 basic skeletons have been isolated, purified, and structurally elucidated. Natural or synthetic triterpenoid compounds have been proven to have superior biological activities against various types of cancers, inflammations, and metabolic syndromes via related signaling pathways with the help of preclinical experiments. However, their inherent systemic toxicity and poor water solubility cause different levels of side effects and low bioavailability; inevitably, they possess several limitations for clinical trials as well as for applications in functional foods, pharmaceuticals, cosmetics domains, and similar fields. Therefore, it is urgent to study the pharmacokinetics profile, metabolic pathways, and mechanism of action and also to improve their bioavailability by combining them with chitosan, cyclodextrin, etc. or reducing the molecular particle size. This chapter summarizes the advanced knowledge about triterpenoids, mainly those stemmed from medicinal plants, in relation to the following aspects: bioactivities whether animal or human, bioavailability, metabolism, safety, side effects, and application in distinct fields especially dietary supplements. Moreover, structural formulas of common basic skeleton and the compounds mentioned in the context, market products, and patents are also discussed.

Keywords

Triterpenoids · Phytochemicals · Pharmacokinetics · Plant · Clinical · Bioavailability · Dietary

13.1 Introduction

Naturally occurring triterpenoids, also known as triterpenes, form a pivotal part of terpenoids. They are regarded as the largest class of plant secondary metabolites, characterized generally by six isoprene units, i.e., 30 carbon atoms, and classified into mono-, di-, tri-, tetra-, penta-, and hexa-cyclic compounds, among which tetra- and pentacyclic are more popular (Cargnin and Gnoatto 2017). Historically, in 1788 Lowitz first extracted betulin from birch tree bark, although it was named by Mason in 1831. Later, researchers in succession roughly separated the representative triterpenoids such as oleanolic acid, ursolic acid, and glycyrrhetinic acid (GA) in the nineteenth century. In 1887, Vesterberg isolated pure α- and β-amyrin successfully and assigned molecular formula to them (Dev 2018). Subsequently, scholars focused on the structural chemistry of triterpenoids. In fact, before the mid-twentieth century, substantial progress in the field of triterpenoids was not obtained. After Ruzicka (1953) put forward the biogenetic isoprene rule theory and with the development of spectroscopy and extraction technology, separation, purification, and structural elucidation of triterpenoids are no longer a stiff problem. To date, thousands of

scholars have isolated, purified, and structurally elucidated more than 20,000 triterpenoid compounds from a variety of plant species worldwide (Wang et al. 2014).

As bioactive phytochemicals, triterpenoids are widely distributed in multifarious plant species. They contain free acids or aglycones, and are synthesized based on the cytosolic mevalonate reaction pathway similar to that of sterols (Ikeda et al. 2008). Briefly, isopentenyl-diphosphate (IPP) generated from acetyl-coenzyme A (acetyl-CoA) combines with its isomer dimethylallyl pyrophosphate (DMAPP), and then they together convert into farnesyl-diphosphate (FPP) through several reaction steps including acetylation, reduction, and phosphorylation in the presence of related enzymes. Next, using squalene synthase, FPP gets converted into squalene which further transforms into 2,3-oxidosqualene by oxidosqualene cyclases. Finally, cyclization of 2,3-oxidosqualene forms parental heterogeneous scaffolds such as lanosterol, cycloartenol, dammarenediol, α-amyrin, β-amyrin, etc. with modified functional groups or rearranged basic skeletons, leading to structural diversity. To date, more than 100 structures have been distinguished (Mullen et al. 2016). In fact, dating back to ancient time, numerous plants have been widely used for remedial purpose. As early as 1578, Shizhen Li compiled *Compendium of Materia Medica*, recording morphology, flavor, and usage of approximately 1892 medicinals, including triterpenoid-rich plants, such as *Centella asiatica*, *Glycyrrhiza uralensis*, *Alisma orientale*, *Momordica charantia*, *Cimicifuga foetida*, *Ganoderma lucidum*, *Panax ginseng*, and *Poria cocos*, which involved Alismataceae, Apocynaceae, Araliaceae, Betulaceae, Compositae, Cruciferae, Gentianaceae, Labiatae, Leguminosae, Myrtaceae, Oleaceae, Polygalaceae, Ranunculaceae, Rubiaceae, Solanaceae, and more families. Some literatures have introduced novel triterpenoids based on their sources from families to genes.

In-depth research on the synthesis and preclinical or clinical experiments of triterpenoids demonstrates that botanical triterpenoids and their derivatives are effective in the treatment of inflammation, obesity, diabetic mellitus, atherosclerosis, and hyperlipidemia, have tumor-multiplication inhibiting effects, and so on. The corresponding action mechanisms may be related to nuclear factor-kappa B (NFκB), adenosine monophosphate (AMP)-activated protein kinase (AMPK), phosphoinositide 3-kinase (PI3K), peroxisome proliferator-activated receptor alpha (PPARα), mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription 3 (STAT3), and other signaling pathways. So far, there are over 300 new derivatives of triterpenoid acids that have been synthesized at Dartmouth College, USA (Liby et al. 2007). It has been verified that efficacy and bioavailability of 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO) and its homologous compounds are stronger than those of triterpenoid acids. Nevertheless, undesirable physicochemical properties such as low aqueous solubility, poor bioavailability, and low permeability limit therapeutic application and clinical experiment of triterpenoids. The absolute bioavailability of oleanolic acid was only 0.7% for oral doses of 25 and 50 mg kg^{-1} (Gao et al. 2017). Naturally occurring triterpenoids without having prominent bioactivities at low concentration and without enough content to carry out clinical experiments are not suitable candidates for the same.

Consequently, it is indispensable to either synthesize novel compounds by altering chemical groups or reduce the oral drug size to nanometer scale (Wang et al. 2013b).

In view of the curative potency, triterpenoid-rich plants or their major active constituents have been extensively utilized in dietary supplements, medicine, and cosmetics fields. In China, oleanolic acid has been used as over-the-counter drugs for the treatment of chronic or acute hepatitis. Glycyrrhizic acid is used extensively as a sweetener in beverages and chewing tobacco. This chapter introduces updated knowledge of triterpenoids with regard to their chemical structures, bioavailability, metabolism, bioactivities, clinical experiment, application, safety, and patent aspects.

13.2 Bioactive Constituents

According to incomplete statistics, more than 100 distinct triterpenoid scaffolds including squalene, limonoids, cucurbitane, cycloartane, protostane, dammarane, lanostane, fusidane, euphane, friedelane, hopane, lupane, oleanane, gammacerane, ursane, α-amyrin, and β-amyrin compounds in which tetra- and pentacyclic triterpenes make up the majority (Hill and Connolly 2017; Teng et al. 2018) have been recognized.

Tetracyclic triterpenoids consist of cucurbitane, cycloartane, protostane, dammarane, lanostane, fusidane, euphane-type, etc., and they are found in various plants; however, different types have targeted plant sources. Cucurbitane-type largely stems from Cucurbitaceae such as *Siraitia grosvenorii*, *Momordica charantia*, and *Hemsleya amabilis*, for which the main bioactive compounds include cucurbitacins and mogrosides. Structural characterizations of these compounds indicate the presence of a variety of oxygen-containing functional group substitution at different positions (Chen et al. 2005). Cycloartane-types are regarded as the characteristic components of *Cimicifuga* and are widely distributed in various families, for example, Ranunculaceae (*Thalictrum*), Leguminosae (*Astragalus*), Meliaceae (*Trichilia casaretti*), Juncaceae (*Juncus effusus*), and so forth. Lanostane-type triterpenoids contain pachymic acid and ganoderic acid found in *Poria* and *Ganoderma* genera, whereas dammarane-type triterpenoids contain ginsenosides and ganoderiols existing in *Panax* genus. Protostane-type, as a stereoisomer of dammarane-type, is mainly found in *Alisma orientale*. Friedelane, lupane, oleanane, gammacerane, ursane, α-amyrin, β-amyrin, and hopane-type consist of pentacyclic triterpenoids, which are distributed ubiquitously and abundantly. Their active compounds arouse researchers' enormous interest and made them lucubrate in vivo or in vitro trials.

Figure 1 shows the parts of structural formula of bioactive constituents and their skeletons studied herein, concretely, including squalene; cucurbitacin B, cucurbitacin E, and saponin mogroside V (cucurbitane); celastrol and its methylether-pristimerin (friedelane); pachymic acid, eburicoic acid, and ganoderic acid A (lanostane); ganoderiol F (ganoderiol F); nimbolide and limonin (limonoid); β-boswellic acid, 11-keto-β-boswellic acid (KBA), and acetyl-11-

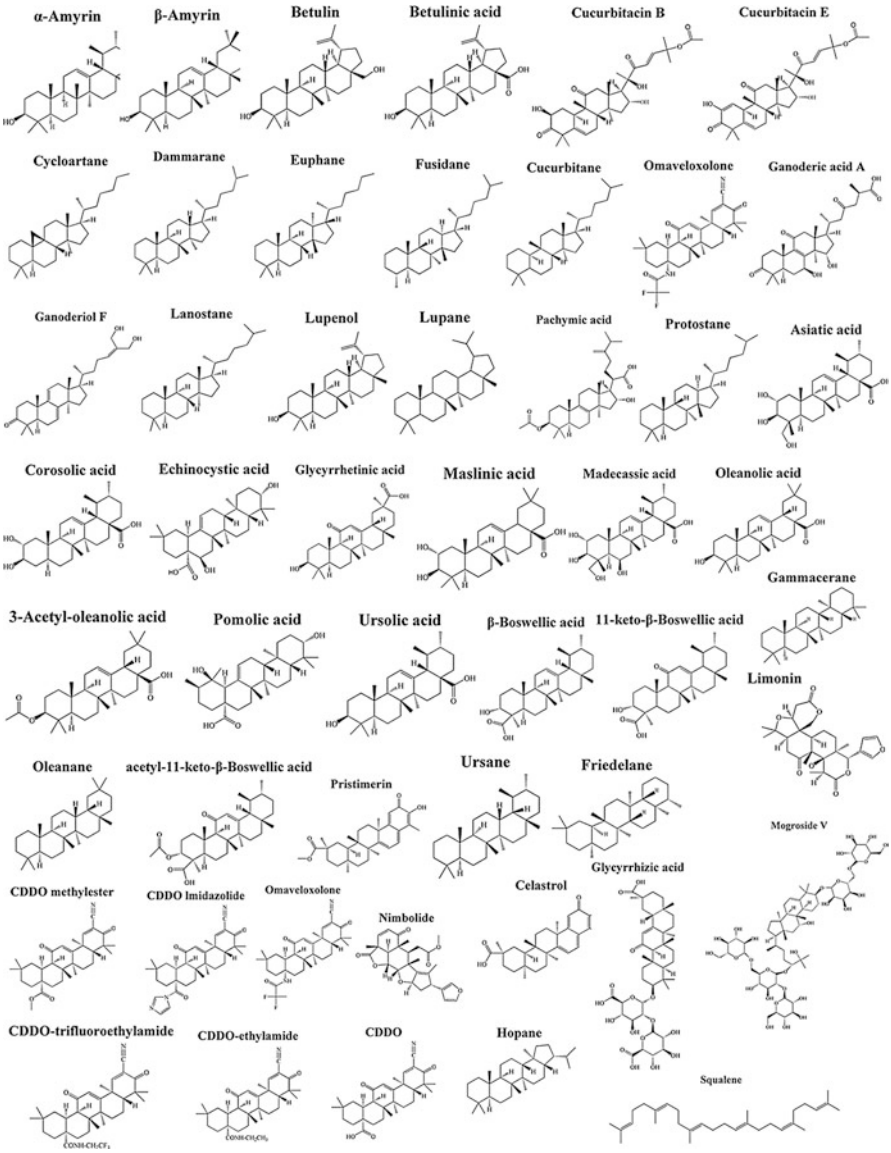

Fig. 1 Parts of structural formula of bioactive constituents described in this chapter

keto-β-boswellic acid (AKBA); lupeol, betulin, and betulinic acid (lupane); oleanolic acid, 3-acetyl-oleanolic acid, CDDO, CDDO-ethyl amide (CDDO-EA), CDDO imidazolide (CDDO-Im), CDDO methyl ester (CDDO-Me), CDDO-trifluoroethyl amide (CDDO-TFEA), omaveloxolone, GA, glycyrrhizic acid, echinocystic acid, and salaspermic acid (oleanane); and corosolic acid, maslinic

acid, ursolic acid, centellosides (asiatic acid and madecassic acid), and pomolic acid (ursane).

13.3 Bioavailability and Metabolism

When a drug enters the body through intravenous, intramuscular, subcutaneous, oral, mucosal, cutaneous, or transdermal route, it undergoes four processes of absorption, distribution, metabolism, and excretion (ADME), which are key physiological properties of pharmacokinetics. Pharmacokinetics is the discipline that involves the study of the efficacy of ADME by measurements, analysis, and prediction of the changes of drug concentrations in human body plasma by using the mathematical principles. In contrast, pharmacodynamics refers to the action mechanisms of drug effects on the organism, and both play a critical role in drug delivery system (Heller et al. 2018). However, various factors such as the physical and chemical properties of the drug, the given dosages and concentration, the route of administration, and the binding ability to plasma proteins affect the actual efficacy of the drug in the body. Rectal administration can avoid several aforementioned influence factors profited from its three transmission routes. Roughly, first, it is directly absorbed into the large circulation by the middle vein, inferior vein, and anal veins of the rectum without passing through the liver so as to avoid the first pass effect; second, the upper rectal vein enters the liver through the portal vein and then participates in the systemic circulation after metabolism; and third, the rectal lymphatic system also absorbs some drugs. The three pathways do not pass through the stomach and small intestine, thus avoiding the effects of acidic and alkaline digestive enzymes on the drug and also reducing the stimulation of the drug to the gastrointestinal tract. Therefore, rectal administration significantly improves the bioavailability of the drug (Van Hoogdalem et al. 1991; Taha et al. 2004). Moreover, Van De Waterbeemd and Gifford (2003) reported that poor ADME properties and target-organ toxicity (together for short ADME/T) are the main reasons for failure of clinical research and commercialization of triterpenoids. According to literature, the budget for developing a candidate drug should exceed $800 million (Yang et al. 2012). During the period from 2000 to 2010, out of 808 drug candidates developed by four major pharmaceutical companies, 356 compounds (44%) failed to progress into clinical studies due to their toxicity. Therefore, it is overwhelming to systematically study the pharmacokinetic and pharmacodynamics behavior of triterpenoids for better facilitating further research.

13.3.1 Bioavailability

The US Food and Drug Administration (FDA) states that bioavailability is the rate and extent to which the therapeutic moiety is absorbed and becomes available to the site of drug action (Holst and Williamson 2008). In other words, bioavailability is the

percentage of drug dose administered by any extravascular route that gets absorbed into the systemic blood circulation (Toutain and Bousquet-Mélou 2004). In general, bioavailability can be categorized into absolute and relative values, whose value contrasted with intravenous administration presumed as 100%, all of them assessed by maximum plasma concentration (C_{max}), the half-life in plasma ($t_{1/2}$), the time required to reach that concentration (t_{max}), the area under concentration-time curve (AUC), and related parameters. The study of medicinal plants has gradually deepened from the physicochemical properties and the level of pharmacological action of drug components to the level of drug bioavailability, which decides therapeutic effects. Factors affecting bioavailability include physicochemical properties (lipophilicity, solubility, and ionization), membrane permeation, presystemic metabolism, and first-pass clearance.

Several intrinsic physical characteristics of triterpenoids affect their bioavailability, which include low water solubility (<1 μg mL^{-1}), poor gastrointestinal absorption, reduced systematic exposure, and extensive metabolic clearance. The aqueous solubility of triterpenoids is generally low, roughly up to 0.02 μg mL^{-1}, e.g., lupeol, oleanolic acid, and betulinic acid. The biopharmaceutical classification system classified common oral solid preparations into four types in consideration of solubility, gastrointestinal permeability, and dissolution. Most triterpenoids or plant extracts belong to the fourth category, that is, low solubility and low permeability. Table 1 summarizes the bioavailability of triterpenoids. Jeong et al. (2007) evaluated the pharmacokinetics of oleanolic acid, indicating that its absolute oral bioavailability in Sprague–Dawley (SD) rats was only 0.7% for oral doses of 25 and 50 mg kg^{-1}. Zhang et al. (2012) studied the pharmacokinetics of celastrol showing that its absolute oral bioavailability in SD rats was 17.06% for oral doses of 1 mg kg^{-1}. Sasaki et al. (2003) investigated the pharmacokinetics of glycyrrhizic acid solution, reporting that its absolute oral bioavailability in SD rats for extravascular doses of 30 mg kg^{-1} was lower (0.25%) for oral administration and higher (20.2%) for nasal administration. Tremendous research efforts have been devoted to improve their bioavailability. At present, researchers mainly concentrate on the following six delivery systems: (1) non-covalent complex with hydrophilic cyclodextrins (CD); (2) nanoparticulate drug delivery; (3) chemical modification, prodrugs; (4) self-nanoemulsifying/microemulsifying drug delivery system (SN/MEDDS); (5) supercritical anti-solvent process; and (6) lipid/phospholipid-based delivery system. Of course, universal methods, for instance, hydrogel and submicron emulsions, do exist. Product launching requires a long process. Fourier-transform infrared spectroscopy, Raman spectroscopy, thermodynamic analysis, differential scanning calorimetry, phase solubility, etc. are employed to determine that the effectiveness of the complex is superior to that of the guest, followed by in vitro experiments, animal experiments, and clinical experiments to verify the activity and safety of the complex.

CDs are a type of cyclic oligosaccharide, which are formed head-to-tail by linking 6–12 D-glucopyranose residues through α-1,4-glycosidic bonds, and most of α-, β-, and γ-CDs severally contain six, seven, and eight units. Taking advantages of their structural characterization results indicating external hydrophilic group with

Table 1 Bioavailability of triterpenoids

Compound	Mode of administration	Experimental model	Biological utilization rate	References
Asiatic acid	Oral (20 mg kg^{-1})	SD rat	16.25%	Yuan et al. (2015)
Celastrol	Oral (1 mg kg^{-1})	SD rat	17.06%	Zhang et al. (2012)
25(R)-OCH_3-PPD	Caudal vein (2 mg kg^{-1})	SD rat	0.14 ± 0.19%	Shao et al. (2017)
25(S)-OCH_3-PPD	Caudal vein (2 mg kg^{-1})	SD rat	28.9 ± 13.9%	Shao et al. (2017)
25(S)-OCH_3-PPD	Oral (10 mg kg^{-1})	SD rat	19.7 ± 7.6%	Wang et al. (2008)
Ganoderiol F	Oral (20 and 50 mg kg^{-1})	Wistar rat	F = 0.105	Zhang et al. (2009)
Ganoderic acid A	Oral (20 mg kg^{-1})	SD rat	8.68%	Cao et al. (2017)
Ginsenoside Rb_1	Oral (180 mg kg^{-1})	SD rat	4.35%	Xu et al. (2003)
Ginsenoside Rg_1	Oral (180 mg kg^{-1})	SD rat	18.40%	Xu et al. (2003)
Mogroside V	Oral (5 mg kg^{-1})	SD rat	8.73 ± 1.46%	Luo et al. (2016)
Nimbolide	Oral (50 mg kg^{-1})	SD rat	3.06%	Mahamuni et al. (2018)
Oleanolic acid	Oral (25 and 50 mg kg^{-1})	SD rat	0.7%	Jeong et al. (2007)

hydrophobic cavity inside, CDs were selectively incorporated with triterpenoids. This finally resulted in the formation of non-covalent inclusion complexes, which led to the improvement in the stability, water solubility, and bioavailability of phytoconstituents (Claude et al. 2004). Yang et al. (2011) prepared nimbin wrapped in β-CD or its derivatives to assemble nimbin/β-CD conjugates which acted as potential candidates for healthcare applications by virtue of satisfactory aqueous solubility and high thermal stability. Xiao et al. (2016) used oleanolic, echinocystic, ursolic, and betulinic acid substrates and synthesized a batch of pentacyclic triterpenoids/α-CD complex, which exhibited increased lipophilicity and improved anti-hepatitis C virus (HCV) activity, whose mechanism depended on preventing virus entry at post-binding step. López-Miranda et al. (2018) explored elaborately the complexation behaviors of oleanolic and maslinic acid complexed with α-,β-,γ-, hydroxypropylated (HP)-α-, HP-β-, and HP-γ-CDs by analyzing thermodynamic parameters, complexation constant, complexation efficiency, and solubility under different pH and temperature conditions. This method has been implicated in oleanolic, betulinic, and glycyrrhizic acid, GA, pedunculoside, and boswellic acids (Lima et al. 2016).

Nanoparticulate drug delivery systems include solid lipid nanoparticles, nanosuspension, nanoencapsulations, and nanoemulsion. Sanna et al. (2015)

prepared celastrol-loaded poly(ε-caprolactone) nanoencapsulations (CL-NP) by nanoprecipitation method and studied thermal properties, antiproliferative efficacy, and mechanism of action on prostate cancer cells. The results indicated that CL-NPs showed higher cytotoxicity with inhibitory concentration (IC50) <2 μM. Nanosuspension is a submicron colloidal dispersion prepared with excipient substrates via high-pressure homogenization. The carriers include albumin, chitosan, gelatin, and poly-lactic acid which can shrink particle sizes and expand surface area to prolong drug release time and further improve bioavailability. Li et al. (2011) reported that nanosuspension of oleanolic acid is greater than that of pure oleanolic acid in saturation solubility, dissolution rate, and oral bioavailability (six- to seven fold). Cháirez-Ramírez et al. (2015) developed and evaluated the properties of lupeol nanonutraceuticals. Apparently, a nanoemulsion loaded with betulinic acid can be used as an effective delivery system that improves oral bioavailability by enhancing its absorption, reducing its clearance, and prolonging its duration of action. Zhang et al. (2016) reported the development of 25-OCH_3-PPD nanoemulsion based on phospholipid complexes. The phospholipid complex of 25-OCH_3-PPD was prepared and evaluated in vitro and in vivo. Solubility studies showed that the solubility of the phospholipid complex in water (4.9 times) and *n*-octanol (1.4 times) was higher. In vivo pharmacokinetic results are expected to demonstrate improved oral bioavailability of 25-OCH_3-PPD using nanoemulsions prepared from phospholipid complexes.

Prodrugs, as inactive and bioreversible derivatives of active phytochemistry, undergo an enzymatic and/or chemical *in vivo* reaction to release the active parent drug, which is usually designed for targeting delivery through specific membrane transporters (Huttunen et al. 2011). Zhou et al. (2017) reviewed the application, merit, and demerit of prodrugs strategy in triterpenoids, including esters, amides, glycosides, mutual prodrug, polymers, and several natural prodrugs, no matter which one is capable of improving poor drug-like properties from solubility, half-life, site selectivity, and presystem metabolism. Cao et al. (2012) selected peptide transporter1 to synthesize prodrug of oleanolic acid whose oral bioavailability enhanced by 2.04-fold.

SN/MEDDS can be defined as anhydrous homogenous liquid mixtures composed of oil, surfactant, cosurfactant (or solubilizer), and triterpenoids; which spontaneously come into oil/water (O/W) nanoemulsion/microemulsion with droplet size <200 nm after diluting in water under gentle stirring (Patel and Velikov 2011). Xi et al. (2009) selected Sefsol 218 as oil phase, Cremophor EL and Labrasol as primary surfactants, and Transcutol P as cosurfactant and optimized three SNEDDS-oleanolic acid formulations for which ratio of Sefsol 218/Cremophor EL/Labrasol/Transcutol P was 50:25:25:0/50:20:20:10/50:17.5:17.5:15 (w/w), respectively, based on dissolution, stability, and particle size profiles. The relative bioavailability of SNEDDS-oleanolic acid complex showed a 2.4-fold increase; moreover, an increased mean retention time and dissolution in rat plasma was also observed compared to commercial tablet. Yang et al. (2013) optimized the formulation design of SMEDDS-oleanolic acid in which Cremophor EL, alcohol, and ethyl oleate acquired the ratio 35:15:50 (w/w) and pharmacokinetic profiles indicated 5.07-fold

increase in oral relative bioavailability contrasting with oleanolic acid tablet. Shen et al. (2016) prepared akebia saponin D-phospholipid complex (APC) by solvent-evaporation method and further optimized the new formulation for APC-SNEDDS using glyceryl monooleate (type 40), polyoxyl 35 castor oil, diethylene glycol monoethyl ether, and APC severally as oil, surfactant, cosurfactant, and drug in a ratio of 1:4.5:4.5:1.74. Finally, results showed that the lipophilicity of APC enhanced (11.4-fold) and the oral bioavailability of APC and APC-SNEDDS improved significantly to 183.8% and 431.8%, respectively. Qi et al. (2014) screened the most suitable formulation of liquid celastrol-SMEDDS system including surfactant (OP-10) 60%, co-surfactant (Transcutol P) 15%, and oil phase (ethyl oleate) 25%, through evaluating solubility, self-emulsifying grading, droplet size, and ternary phase diagram parameters, and selected microcrystalline cellulose MCC KG 802 as adsorbent to prepare celastrol-SMEDDS dispersible tablets. Whether liquid celastrol-SMEDDS or its dispersible tablets were used, relative bioavailability was, respectively, 569 ± 7.07% and 558 ± 6.77% and significantly enhanced compared to 0.4% sodium carboxymethylcellulose suspension.

Supercritical anti-solvent process is a micronization technology to make triterpenoid particles reach micronic or nanometric scale. Yang et al. (2012) administered ursolic acid microparticles (100 mg kg^{-1}) in male Wistar rats and found that both the dissolution rate and absorption increased. Sui et al. (2012) prepared micronized glycyrrhizic acid and administered 250 mg kg^{-1} in Wistar male rats to compare the difference of pharmacokinetics. Results showed that the maximum saturation concentration was fivefold higher than that of pure glycyrrhizic acid and the pharmacokinetics properties improved significantly. Of course, other technologies for microparticle preparation including co-melting, dissolution, spray freeze-drying, and solvent evaporation also exist (Tong et al. 2011).

Lipid/phospholipid-based delivery system, for short liposomes/phytosomes, uses lipid or phospholipid as vesicles complexed with active constituents. Further, they together dissolve in non-polar solvents to obtain cell-like structure. Celastrol was dissolved in bits of anhydrous ethanol after being mixed with soy phosphatidylcholine. First, magnetic stirring was carried out for 3 h, and then the solvent was removed by rotary evaporation. The celastrol-phospholipid complex was formed, which was stored in desiccators. The celastrol phytosomes were prepared by brief sonication of the celastrol-phospholipid complex by adding deionized water. Compared to crude celastrol, AUC of celastrol phytosomes exhibited obvious fourfold increase, with fivefold increase in C_{max} (Freag et al. 2018). Boswellic acids were dissolved in bits of dichloromethane after being mixed with soy phosphatidylcholine. First, magnetic stirring was carried out for 2 h, and then the solvent was removed by rotary evaporation. The as-formed boswellic acid–phytosome complex was kept in desiccators. The method improves pharmacokinetics and anti-inflammation activity of boswellic acids (Sharma et al. 2010). Thin film dispersion–sonication method was used to prepare polyvinylpyrrolidone-K30-modified oleanolic acid liposomes. First, oleanolic acid was dissolved in little amount of methylene chloride, then it was mixed with soy lecithin and cholesterol, and then the solvent was removed by rotary evaporation to form film. The liposomes solution was

disrupted and freeze-dried with solution containing sodium deoxycholate, phosphate buffered saline, and 1% polyvinylpyrrolidone-K30, and finally the product was obtained successfully. The relative bioavailability of novel liposomal formulation enhanced by 6.08-fold; moreover, its absorption and stability in gastrointestinal tract increased (Liu et al. 2017b).

13.3.2 Metabolism

After a drug gets distributed ubiquitously in the human body, a series of metabolic reactions occurs under the influence of various enzymes and body fluid environment, resulting in changes in the chemical structure of the drug, and then it displays enhanced water solubility which is in favor of excretion. Owing to the structure of the material and the influence of the efflux protein, the triterpenoids are poorly absorbed in the intestinal tract and have low bioavailability. Triterpenoid is retained in the intestinal flora for a long time, and then little amount is absorbed by the bacterial group after hydrolysis. Nevertheless, owing to the complexity of internal environment and limitation of in vitro research techniques which cannot be extrapolated to the human body, only a few literature reports are available. Table 2 summarizes the metabolic pathways and metabolites of triterpenoid.

Metabolism can be divided into the following three phases: phase I includes hydrolysis, oxidation, and reduction reactions catalyzed by cytochrome P450 (CYP) superfamily; phase II points out at conjugation reaction between active compounds or their metabolites with some substrates; and phase III involves drug transporters placed in gastrointestinal tract, kidney, and epithelial and endothelial cells of the liver etc. and facilitates metabolites transport across blood–brain barrier. The intestinal and hepatic metabolism is called by a joint name presystemic metabolism. Metabolism in the liver is mainly accomplished by liver microsomes. To date, in vitro human liver models include cytosol, S9 fraction, supersomes, microsomes, transgenic cell lines, primary hepatocytes, liver slices, perfused liver, and others. All of them have their own highlights and weaknesses (Brandon et al. 2003).

Kimura et al. (2008) found that the elimination of glycyrrhizic acid from the liver may be one of the factors leading to low oral bioavailability by comparing the AUC of mice administered with the same dose via hepatic portal vein and intravenous injection. They also found that oral administration of glycyrrhizic acid in mice without intestinal flora did not detect GA in plasma. It was thus concluded that most of glycyrrhizic acid was hydrolyzed into GA in the presence of intestinal flora in the small intestine; and GA is a non-ionic molecule with strong liposolubility and it easily enters into the blood circulation through the intestinal wall. Jeong et al. (2007) investigated the absorption and metabolism of oleanolic acid by Caco-2 cell monolayer and liver microsome model. Studies showed that oleanolic acid gets metabolized by a variety of enzymes present in the intestine and liver, in particular CYP isozymes, such as CYP3A. Liao et al. (2005) observed the in vivo process of ursolic acid in rats and found that even though the dosage was high, the concentration of ursolic acid in the blood plasma was very low. It could thus be predicted that

Table 2 Metabolism of triterpenoids

Compound	Types	Metabolite	Metabolic pathways	References
25 (R)-OCH_3-PPD	CYP3A4	25-OH-PPD	Hydroxylation, dehydrogenation, and *O*-demethylation	Zhang et al. (2014a)
Asperosaponin VI	Intestinal flora	Hederagenin	Hydrolysis	Zhang et al. (2014b)
Ganoderiol F	Intestinal flora	Ganodermatriol	Hydrolysis	Zhang et al. (2009)
Ginsenoside Rb_1	Intestinal flora	Rd, Rg3, or F2, Rh2	Hydrolysis	Qian et al. (2006)
Glycyrrhizic acid	Intestinal flora	GA	Hydrolysis	Murata et al. (2010)
Lancemaside A	Intestinal flora	Echinocystic acid	Deglycosylated	Joh et al. (2012)
Mogroside V	Intestinal flora	Mogrol	Deglycosylated	Luo et al. (2016)
Soyasaponin I	Intestinal flora	Soyasaponin B	Hydrolysis	Hu et al. (2004)
Saikosaponin C	Intestinal flora	Prosaikogenin E1, E2, and E3, saikogenin E	Hydrolysis	Yu et al. (1997)

ursolic acid is widely distributed in the tissues and less distributed in the blood stream. Moreover, it may also be attributed to the decrease of bioavailability after metabolism through the intestine or liver. Some experiments showed that the metabolism of ursolic acid in the rat liver microsome incubation system treated with CYP3A inducer dexamethasone was significantly faster than that in the blank control group. After 30 min of metabolism, substitution of ursolic acid in the liver microsome incubation system was significantly different from that in the blank control group ($P < 0.01$). Furthermore, the classical CYP2B inducer phenobarbital and CYP1A inducer β-naphthoflavone were used as probes to pretreat the rats, respectively, and there was no significant difference in metabolic capacity between the two groups ($P > 0.05$), indicating that ursolic acid was mainly mediated by CYP3A in liver microsomes. The obvious homology of CYP3A cDNA sequences in liver and intestine indicates that the metabolism of ursolic acid by intestinal enzyme CYP3A may be one of the reasons for its low oral bioavailability.

Panax ginseng, whose active components include ginsenosides, is used to treat diabetes, insomnia, debility, aging, and sexual inadequacy from ancient century. As one of the popular Chinese herb medicines, its safety is relatively credible; however, they lack scientific theoretical data support. Thus, increasing research efforts should be focused upon to carry out systematic explorations on pharmacokinetics and pharmacodynamics of ginsenosides and their metabolism prior to animal experiments. Akao et al. (1998) compared cumulative contents and feces of germ-free and infected with *Eubacterium* sp. A-44 rats and found that ginsenoside Rb_1 could transform into compound K. Lai et al. (2009) investigated pharmacokinetic profiles

and metabolic pathways of 20(S)-ginsenoside Rh_1. Results showed that its absolute bioavailability was only 1.01%. Moreover, its metabolic pathways include deglycosylation in intestinal bacteria and hydration reaction in gastric; thus, it is reasonable to suspect that presystemic metabolism causes poor bioavailability. At present, more than 20 ginsenosides are deglycosylated by intestinal bacteria to active forms through oral conduction.

Limonin is one of the most representative limonoids, which belong to highly oxygenated C13α-triterpenoids, with intense bitterness and existing in citrus seeds. In fact, limonin was isolated in 1841, and till 1960, its exact structure was determined by the five teams working together. Furthermore, several hundred congeners were separated. Limonin can prevent osteoporosis, intestinal carcinogenesis, hepatocarcinogenesis, hypertension, etc. The in vitro and in vivo trials were carried out to expound the biotransformation and metabolic profile of limonin, i.e., possible metabolites and metabolic pathways. The mixture of limonin and rat's liver microsome incubation and the urine and blood samples were collected at 6, 12, and 24 h of SD rats after administering 80 mg kg^{-1} limonin. The samples were subjected to high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry analysis. Results indicated that five metabolites were identified and formed through reduction and hydrolysis pathways (Liu et al. 2018a).

13.4 Bioactivities

13.4.1 Crude Extractions

Leaves, roots, and stems of plants are utilized for health-promoting purpose from time immemorial including fungi, ferns, monocots, dicots, animals, and marine life. Researchers have isolated and identified large quantity of triterpenoids which were intended to be the major active components for curative effect. To date, oleanolic acid exists in more than 1600 plant species; moreover, ursolic acid is separated from up to 120 plant species. In fact, crude extractions have more potent activities by virtue of collaboration of different compounds. In recent years, crude extracts of triterpenoids have been reported to possess various biological and pharmacological properties such as anti-diabetes, anti-fungal, cytotoxic, liver-protecting, anticancer, chemopreventive, anti-allergic, immunomodulatory, hemolysis, immunostimulatory, and anti-inflammatory activity. Table 3 summarizes the bioactivities (limited to rodents' experiments) of crude extracts from triterpenoid-rich plants.

Momordica charantia (Cucurbitaceae), commonly known as bitter melon, is widely cultivated as a subtropical vegetable crop in Asia and has been used to treat shanghuo, toothache, diabetes mellitus, diarrhea, and furuncle in China. More than 50 triterpenoids have been isolated, among which representative triterpenoids are cucurbitane-type which possess anticancer and antidiabetic activities as verified scientifically (Wang et al. 2017). Pitchakarn et al. (2010) studied the effect of 80% ethanol extracted from leaves of *M. charantia* on prostate cancer in vivo and in vitro. PLS10 cell line was selected to carry out cytotoxicity, growth inhibition,

Table 3 Bioactivities of crude extractions

Plant origin	Diseases	Animal	Dose	Results	References
Antrodia camphorata	Ergogenic and antifatigue	Institute of Cancer Research (ICR) strain mice	50 or 200 mg kg^{-1}	↑Physical fatigue and exercise performance	Huang et al. (2012)
Bacopa monnieri	Diabetes mellitus	Wistar albino rats	300 mg kg^{-1}	↑Cellular antioxidant defense Protect lipid peroxidation	Ghosh et al. (2011)
Bauhinia racemosa	Inflammation	Wistar albino rats Swiss albino mice	50, 100, 200 mg kg^{-1}	Against acute and chronic phases of inflammation	Gupta et al. (2005)
Boswellia serrata (resin)	Hepatotoxicity	Swiss albino mice	125, 250, 500 mg kg^{-1}	↑Altered liver enzyme activities, oxidative stress markers, liver histopathological features	Barakat et al. (2018)
Catharanthus roseus	Wound healing	SD rats	100 mg kg^{-1}	↑Wound contraction and tensile strength	Nayak and Pinto Pereira (2006)
Cestrum nocturnum	Diabetes mellitus	Wistar rats	200 or 400 mg kg^{-1}	↑Biochemical parameters	Kamboj et al. (2013)
Eugenia jambolana	Diabetes mellitus	C57BL/6 mice	100 mg kg^{-1}	↓Hepatic gluconeogenesis	Li et al. (2017b)
Ganoderma lucidum	Benign prostatic hyperplasia	SD rats	1.5 and 15 mg kg^{-1}	↓5α-reductase activity	Liu et al. (2007)
Hippophae rhamnoides	Urinary dysfunction	SD rats	10 μg mL^{-1}	↓Bladder specimen contraction	Shimoda et al. (2017)
Ilex kudingcha	Atherosclerosis	C57BL/6J $ApoE^{-/-}$ mice	50 and 100 mg kg^{-1}	↓Aortic sinus lesions	Zheng et al. (2015)

Japanese apricot	Esophageal cancer	Severe combined immunodeficiency and CB-17 mice	2.5 mL kg^{-1}	↓Doses of anticancer drug ↑Potentiate anticancer effect	Yamai et al. (2010)
Lactuca sativa	Analgesic and inflammation	NMRI mice and Wistar rats	1, 3, 6 g kg^{-1} or 1, 2, 4 g/kg	↓Pain and inflammation	Sayyah et al. (2004)
Ligustrum	Nociception	SD rats	0.1, 0.25, 1 g kg^{-1}	↓Cyclooxygenase-2 activity ↓Microvascular permeability	Wu et al. (2011)
Mallotus peltatus	Inflammation	Wistar albino rats	200 or 400 mg kg^{-1}	Relieve paw edema	Chattopadhyay et al. (2002)
M. charantia	Prostate cancer	Athymic nude mice	0.1%, 1%(w/w)	Anti-invasion	Pitchakarn et al. (2010)
M. charantia	Liver injury	Kunming mice	250, 500, 750 mg kg^{-1}	↑Antioxidative capacity Protect liver mitochondrion	Deng et al. (2017)
M. charantia	Diabetes mellitus	ICR mice	0.84, 1.68 mg kg^{-1}	↑Insulin sensitivity	Han et al. (2018)
Olea europaea	Cardiovascular	SD rats	60 mg kg^{-1} i.p.	Negative inotropic	Somova et al. (2003)
Potentilla discolor	Diabetes mellitus	Wistar rats	501 mg kg^{-1}	↑Lipid metabolites	Zhang et al. (2010)
Xanthoceras sorbifolia	Alzheimer's disease	Kunming mice	1.33, 4, 12 mg kg^{-1} or 0.93, 2.8, or 8.4 mg kg^{-1}	Oxidative stress and synaptic damage Cholinergic system deficiency	Ji et al. (2017)
Wedelia chinensis	Prostate cancer	Athymic (nu/nu) nude mice	4 or 40 mg kg^{-1}	↓Androgen receptor	Tsai et al. (2009)

invasion, and migration assays. Moreover, athymic nude mice were given extract solution (0.1% or 1%) for 3 weeks to verify the metastasis assay. Deng et al. (2017) studied the effect of water extraction from fruits of *M. charantia* on liver injury in vivo and in vitro. In the beginning, researchers detected the main components of *M. charantia* extraction which included polysaccharide (27.92 g/100 g), protein (15.8 g/100 g), phenolic (0.04 g/100 g), and total saponin (0.48 g/100 g). 72 male Kunming mice were purchased and randomized into six groups administered with intragastrical *M. charantia* extraction 250, 500, and 750 mg kg^{-1} and vitamin C 250 mg kg^{-1} as positive control, and the rest were normal group and restraint stress model group. After 7 days, all the mice were dissected and their blood sample and liver collected. Furthermore, their pathological structure, biochemical analysis, protein expression, and conventional data were studied. Results displayed that *M. charantia* extraction could protect against liver injury in mice subjected to restraint stress by improving antioxidative capacity and protecting the liver mitochondrion. Han et al. (2018) adopted C2C12 myoblasts and ICR mice to study the insulin sensitivity and action mechanisms of four cucurbitane triterpenoids from *M. charantia* fruits' ethanol extract. Mice were assigned into control, C2 (0.84, 1.86 mg kg^{-1}), and positive control rosiglitazone (1.35 mg kg^{-1}) groups; subsequently, epididymal adipose tissue, quadriceps skeletal muscle, liver, and pancreas were collected to conduct glycogen assay and routine testing. The phenomenon that C2 increased glucose uptake into skeletal muscle via insulin receptor substrate-1 activation showed that cucurbitane triterpenoids can be deemed as insulin sensitizer.

Boswellia serrata belongs to Burseraceae family and is cultivated in far-ranging tropical and sub-subtropical regions. Its resin emits intense fragrance; thus it has been used as the raw ingredient of incense, balm, and myrrh long time ago, and its medicinal value has also been recorded in many ancient books, early dating back to 1500 BC in *Ebers Papyrus*. Furthermore, in Indian Ayurveda, there are descriptions about the treatment of arthritic diseases, Crohn's disease, asthma, and inflammation ailments. In China, it is usually used to repair skin damage such as bruises and infected sores. Modern medicine has confirmed that *B. serrata* gum resin extract possesses anti-inflammation, wound healing, immunomodulatory, anti-arthritic, antimicrobial, and neuroprotective activity, possibly profited from boswellic acids which contain more than 12 different compounds, among which KBA and AKBA acquired significant pharmacological attention. Barakat et al. (2018) explored the protective effect of boswellic acids which was bought directly in the form of *B. serrata* extract tablets (covered 65% boswellic acids) on doxorubicin-induced hepatotoxicity model. 40 Swiss albino mice were allocated into five groups ab libitum in control, model, and treatment groups (125, 250, 500 mg kg^{-1}), and finally blood and liver samples were collected. Treatment groups could decrease serum liver enzyme activities and hepatic malondialdehyde levels, upregulate nuclear factor-erythroid-2-related factor 2 (Nrf2) gene expression, and downregulate cleaved caspase-3 gene expression. Along with histopathological examination and DNA fragmentation analysis, integrated results revealed that boswellic acids are useful for protection against hepatotoxicity via impacting Nrf2/heme oxygenase-1 (HO-1) pathway.

13.4.2 Pure Compounds

Owing to the uncertainty on researching structure-activity relationships and biological mechanism actions of the crude extractions, plus the finite content which cannot even meet the requirement of animal experiment in plants, an increasing number of researchers prefer purchasing pure triterpenoids from biological companies, whereas as a result of expensive price, only oleanolic acid, ursolic acid, betulinic acid, and their derivatives are ubiquitous. Therefore, Table 4 summarizes the progress of bioactivities of pure triterpenoids.

Ischemia-reperfusion injury (IRI) refers to the sudden re-acquisition of blood supply to organs or tissues after a period of ischemia and can be divided into two processes, ischemia and reperfusion, which not only fails to restore the function of organs or tissues but also aggravates functional metabolic disorders and structural irreversible damage, on account of the formation of excess free radicals; activation of neutrophils, Kupffer cell, and platelets; endothelial cell injury; and enhanced vascular permeability, nitric oxide, cytokines, nuclear factor, and apoptosis. In clinical practices, IRI occurs in blood circulation disorders such as traumatic shock, surgery procedures, and organ transplantation and exists in many organs such as the myocardium, brain, liver, lung, and retina; in view of the abovementioned considerations, its mechanism is complex and multifactorial. Apolipoprotein E knockout (ApoE-KO) mice received intragastric betulinic acid 50 mg kg^{-1} day^{-1} for 7 days, which proceeded to transient focal ischemia operation in the middle cerebral artery and made the period of ischemia/reperfusion to 2 h/22 h. Pretreatment with betulinic acid could reduce oxidative stress and nitrosative stress, so it was valid to guard against celebral IRI (Lu et al. 2011). Nrf2+/+ (WT) and Nrf2−/− mice bred from pairs of Nrf2+/− (CD1/ICR) mice were administered with CDDO-Im 30 μmol kg^{-1} before 24 h/3 h or after 24 h ischemia. The effect of CDDO-Im on kidney IRI was evaluated thoroughly by comparing the change of renal function, histology, cell, and protein levels with those of normal groups. Pretreatment of CDDO-Im is helpful for protecting against kidney IRI, to a large degree, depending on the characteristic of Nrf2 activator (Liu et al. 2014). Ding et al. (2014) studied the effect of AKBA on mice suffering from middle cerebral artery occlusion and subsequent reperfusion. All the SD rats were divided in three groups based on treatment involving administration of 20 mg kg^{-1} AKBA by intraperitoneal inject. Treatment using AKBA can shrink the infarct volume, remit neuronal damage, and attenuate oxidative stress. Further, integration of Western blot and transfection tests displayed that AKBA possesses neuroprotection by feat of the activation of Nrf2/HO-1 pathway. Xu et al. (2017) and colleagues assessed the efficacy of asiatic acid on hepatic IRI. Male C56BL/6 mice were administered oral dosage of 30 mg kg^{-1} asiatic acid before 1 h vascular occlusion, which was effective to cut down hepatic histopathological damage, apoptotic signaling, and global inflammatory level. Results indicated that hepatoprotection of asiatic acid was collected with proliferator-activated receptor gamma (PPARγ) and nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain-containing 3 (NLRP3). Tong et al. (2018) investigated the effect of celastrol pretreatment (2, 4, and 6 mg kg^{-1}) on myocardial

Table 4 Bioactivities of pure triterpenoids

Compound	Disease	Animal model	Dose	Signaling pathway	References
Arjunolic acid	Acute hepatotoxicity	Wistar albino rats	80 mg kg^{-1}	↓c-Jun-NH2-terminal protein kinase (JNK)/ Bcl-2 and mitochondrial permeabilization	Ghosh et al. (2010)
Asiatic acid	Acute lung injury	BALB/c mice	25, 50, and 100 mg kg^{-1}	↓TLR4/NF-kB	Li et al. (2016b)
Asiatic acid	Cardiac hypertrophy	C57BL/6 mice	10 and 30 mg kg^{-1}	↑AMPKα ↓mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase (ERK)	Ma et al. (2016)
Asiatic acid	Hemodynamic abnormality	SD rats	10 and 20 mg kg^{-1}	Recovering endothelial/inducible nitric oxide synthase (eNOS/iNOS)	Pakdeechote et al. (2014)
Asiatic acid	Sepsis	BALB/c mice	10 and 30 mg kg^{-1}	↑Notch receptor (Notch3) and delta-like ligand (DLL4)	Xiong et al. (2018)
Asiatic acid	Hepatic IRI	C56BL/6 mice	30 mg kg^{-1}	↑PPARγ and NLRP3	Xu et al. (2017)
Asiatic acid	Parkinson's disease	C57BL/6 mice	20, 40, and 80 mg kg^{-1}	↓TLR2 and NF-κB p65 expression	Chao et al. (2016)
Betulin	Alcoholic liver injury	C57BL/6 mice	20 and 50 mg kg^{-1}	Sirtuin 1 (SIRT1)- liver kinase B-1(LKB1)-AMPK	Bai et al. (2016)
Betulin	Kidney injury	SD rats	4 and 8 mg kg^{-1}	TLR4/NF-κB	Zhao et al. (2016)
Betulinic acid	Diabetic nephropathy	SD rats	20 and 40 mg kg^{-1}	AMPK/NF-κB/Nrf2	Xie et al. (2016), Wang et al. (2016b)
Betulinic acid	Hyperglycemia	ICR mice	5 and 10 mg kg^{-1}	Modulate Ca^{2+}-calmodulin dependent protein kinase kinase (CAMKK)-AMPK-cAMP response element-binding protein (CREB)	Kim et al. (2014b)

Betulinic acid	Cerebral IRI	ApoE-KO mice	50 mg kg^{-1}	↓NADPH oxidase subunits (NOX2), (neuronal) nNOS and iNOS ↑eNOS	Lu et al. (2011)
Betulinic acid	Hepatocellular carcinoma	C57BL/6 mice	100 and 200 mg kg^{-1}	p53-$p66^{shc}$/miR-21-Sod2	Yang et al. (2015a)
AKBA	Cerebral IRI	SD rats	20 mg kg^{-1}	Nrf2/HO-1	Ding et al. (2014)
KBA	Cerebral IRI	SD rats	25 mg kg^{-1}	Nrf2/HO-1	Ding et al. (2015)
CDDO	Prostate cancer	C57BL/6 mice	10 mmol kg^{-1}	Akt, NF-kB	Deeb et al. (2011)
CDDO-EA	Amyotrophic lateral sclerosis	G93A SOD1 transgenic familial ALS mice	400 mg kg^{-1}	Nrf2/antioxidant response element (ARE)	Neymotin et al. (2011)
CDDO-Im	Hepatic injury	C57BL/6 mice	1 mg kg^{-1}	Nrf2-kelch-like ECH-associated protein 1 (Keap1)	Reisman et al. (2009)
CDDO-Im	Kidney IRI	$Nrf2^{+/+}$ (WT) and $Nrf2^{-/-}$ mice	30 μmol kg^{-1} (200 μL)	Nrf2	Liu et al. (2014)
CDDO-Me	Diabetes mellitus	C57BL/6J mice	3 mg kg^{-1}	AMPK	Saha et al. (2010)
CDDO-Me	Acute lung injury	BAlB/c mice	0.5 and 2 mg kg^{-1}	↓Mitogen-activated protein kinase (MAPK), NF-κB	Chen et al. (2015)
CDDO-Me, CDDO-EA, CDDO-TFEA	Parkinson's disease	C57BL6 mice	4, 2, 1, and 0.5 μmol	Nrf2/antioxidant response element (ARE)	Kaidery et al. (2013)
Celastrol	Diabetic renal injury	db/m and db/db mice	1 mg kg^{-1}	NF-κB	Kim et al. (2013a)
Celastrol	Cardiac fibrosis	Kunming mice	1 mg kg^{-1}	microRNA21 (miR-21)/ERK	Cheng et al. (2016)
Celastrol	Diabetic nephropathy	SD rats	100, 200, and 500 μg kg^{-1}	TLR4/MyD88/NF-κB	Han et al. (2016)
Celastrol	Hepatocellular carcinoma	Athymic nu/nu female mice	1 and 2 mg kg^{-1}	Signal transducer and activator of transcription 3 (STAT3)/Janus-like kinase (JAK2)	Rajendran et al. (2012)

(continued)

Table 4 (continued)

Compound	Disease	Animal model	Dose	Signaling pathway	References
Celastrol	Ischemic stroke	SD rats	2 and 3 mg kg^{-1}	↓c-Jun N-terminal kinases (JNK) and NF-κB	Li et al. (2012)
Celastrol	Ulcerative colitis-related colorectal cancer	C57BL/6 mice	2 mg kg^{-1}	Epithelial-mesenchymal transition (EMT)	Lin et al. (2016)
Celastrol	Myocardial IRI	SD rats	4 mg kg^{-1}	PI3K/Akt	Tong et al. (2018)
Celastrol	Renal fibrosis	BALB/C mice	1 mg kg^{-1}	↓Cannabinoid receptor 2 (CB2R) expression	Tang et al. (2018)
Celastrol	Incisional pain	SD rats	5, 10, and 20 μg/paw	Sterile α- and armadillo-motif-containing protein (SARM), NF-κB	Chen et al. (2018)
Celastrol	Acute kidney injury	C57BL/6J mice	1 and 2 mg kg^{-1}	↓NF-κB ↑mitochondrial function	Yu et al. (2018)
Corosolic acid	Atherosclerosis	C57BL/6J mice $ApoE^{-/-}$ mice	0.3 mg kg^{-1}	↓Monocyte chemoattractant protein-1 (MCP-1) expression ↓NF-κB	Chen et al. (2012b)
Corosolic acid	Insulin resistance	C57BL/6 mice	10 and 20 mg kg^{-1}	Regulated AMPK activation	Yang et al. (2016)
Cucurbitacin B	Hepatic fibrosis	Albino mice	1 and 5 mg kg^{-1}	↓Signal transducer and activator of transcription 3 (STAT3)	Sallam et al. (2018)
Cucurbitacin E	Hepatic fibrosis	C57BL/6 mice	5 and 10 mg kg^{-1}	Akt-AMPK-mTOR	Wu et al. (2016b)
Cucurbitacin E	Central obesity	C57BL/6 mice	0.25 and 0.5 mg kg^{-1}	JAK- STAT5	Murtaza et al. (2017)
Echinocystic acid	Pain/depression dyad	C57BL/6 mice	5 mg kg^{-1}	Regulating the biogenic amine levels and GluN2B receptors in the hippocampus	Li et al. (2016a)
18β-GA	Liver injury	Wistar rats	25 and 50 mg kg^{-1}	PPARγ/Nrf2 activation	Mahmoud and Al Dera (2015)

18β-GA	Renal injury	BALB/c mice	10, 25, and 50 mg kg^{-1}	↑Nrf2 ↓NF-κB	Wu et al. (2015)
18β-GA	Gastric cancer	K19-C2mE transgenic mice	0.05%	↑miR-149-3p	Cao et al. (2016)
Glycyrrhizic acid	Renal injury	BALB/c mice	25, 50, and 100 mg kg^{-1}	↑Nrf2 ↓NF-κB	Wu et al. (2015)
Glycyrrhizic acid	Non-alcoholic steatohepatitis	C57BL/6 mice	12.5, 25, and 50 mg kg^{-1}	↓Hepatic lipogenesis, inflammation, fibrosis, and lipid metabolism	Wang et al. (2016b)
Glycyrrhizic acid	Non-alcoholic fatty liver disease	C57BL/6 mice	15, 30, and 60 mg kg^{-1}	Regulate genes involved in lipid, glucose homeostasis and insulin sensitivity	Sun et al. (2017)
Glycyrrhizic acid	Bronchial asthma	Balb/c mice	25, 50, and 100 mg kg^{-1}	↓OX40-OX40L and p38 MAPK	Wu et al. (2016a)
Glycyrrhizic acid	Diabetic nephropathy	db/db and db/m mice	15 mg kg^{-1}	AMPK/SIRT1/peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)	Hou et al. (2017)
Limonin	Hepatic IRI	Wistar rats	100 mg kg^{-1}	↓TLR	Mahmoud et al. (2014)
Lupeol	Inflammatory bowel disease	Swiss albino mice	40 mg kg^{-1}	NF-κB	Kasinathan et al. (2018)
Maslinic acid	Cerebral IRI	SD rats	5, 50 mg kg^{-1}	↑NF-κB-mediated GLT-1	Guan et al. (2011)
Maslinic acid	Ischemic stroke	C57BL/6J mice	0.1, 1, and 10 mg kg^{-1}	Regulate Akt-GSK-3β	Qian et al. (2015)
Maslinic acid	Schizophrenia	ICR mice	3, 10, or 30 mg kg^{-1}	Akt-GSK-3β and ERK-CREB	Jeon et al. (2017a)
Maslinic acid	Cardiac hypertrophy	C57BL/6 mice	20 mg kg^{-1}	↓Activation of AKT and ERK	Liu et al. (2018b)
Maslinic acid	Seizure	C57BL/6 mice	20 or 40 mg kg^{-1}	NF-κB p50/65	Wang et al. (2018a)
Maslinic acid	Alcoholic liver disease	BALB/cA mice	10, 15, or 20 mg kg^{-1}	NF-κB p50/65	Yan et al. (2014)

(continued)

Table 4 (continued)

Compound	Disease	Animal model	Dose	Signaling pathway	References
3-Acetyl-oleanolic acid	Non-alcoholic fatty liver disease	SD rats	15, 30, or 60 mg kg^{-1}	AMPK-related pathways	Ou-Yang et al. (2018)
Oleanolic acid	Memory impairment	ICR mice	0.625, 1.25, 2.5, or 5 mg kg^{-1}	Modulating BDNF-ERK1/2- brain-derived neurotrophic factor (CREB)	Jeon et al. (2017b)
Oleanolic acid	Hepatic IRI	SD rats	100 mg kg^{-1}	PI3K/Akt-GSK-3β	Gui et al. (2015)
Oleanolic acid	Hyperlipidemia	C57BL/6 mice	20 mg kg^{-1}	miR-98-5p/PPARγ coactivator-1β	Chen et al. (2017)
Oleanolic acid	Gut atrophy	Female neonatal pigs	50 mg kg^{-1}	TGR5 agonists	Jain et al. (2016)
Oleanolic acid	Cartilage degeneration	db/db mice and C57BL/6J mice	200 mg kg^{-1}	PPARγ	Kang et al. (2017)
Oleanolic acid	Adiposity	C57B6/J mice	50 mg kg^{-1}	Hepatocyte nuclear factor 1b (HNF1b)	Su et al. (2018)
Oleanolic acid	Postmenopausal osteoporosis	C57BL/6 mice	10 mg kg^{-1}	NF-κB	Zhao et al. (2018)
Oleanolic acid	Schizophrenia	ICR mice	3, 10, or 30 mg kg^{-1}	Akt/GSK-3β	Park et al. (2014)
Oleanolic acid	Colitis	C57BL/6J mice	5, 10 mg kg^{-1}	↓Th17 cell differentiation ↑Treg cell differentiation	Kang et al. (2015)
Oleanolic acid	Liver injury	Nrf2-null mice Keap1KD mice	22.5 mg kg^{-1}	Nrf2 activation suppresses Oatp1b2	Lu et al. (2015)
Oleanolic acid	Atherosclerosis	Quails	25, 50, or 100 mg kg^{-1}	↓NADPH oxidase ↑Nrf2 and HO-1	Jiang et al. (2015)
Oleanolic acid	Adipose tissue insulin resistance	SD rats	5 or 25 mg kg^{-1}	Insulin receptor substrate-1/PI3K/Akt	Li et al. (2014b)
Oleanolic acid	Depression	ICR mice	2.5, 5, 10, 20, or 40 mg kg^{-1}	Brain-derived neurotrophic factor (BDNF)-ERK-CREB	Yi et al. (2014)
Oleanolic acid	Allergic asthma	Female BALB/c mice	2 and 20 mg kg^{-1}	↓GATA-3, retinoic acid-related orphan receptor γt (RORγt)	Kim et al. (2014a)

Oleanolic acid	Hepatocellular carcinoma	BALB/c mice	75 or 150 mg kg^{-1}	ERK-p53-mediated cell cycle arrest and mitochondrial-dependent apoptosis	Wang et al. (2013a)
Oleanolic acid	Diabetic nephropathy	OLETF and LETO rats	100 mg kg^{-1}	Endoplasmic reticulum stress reduction	Lee et al. (2015)
Oleanolic acid	Cholestasis	C57BL/6 mice	5, 10, 20 mg kg^{-1}	Nrf2-mediated upregulation of Mrp2, Mrp3, and Mrp4	Chen et al. (2014)
Oleanolic acid	Chronic cyclosporine nephropathy	ICR mice	25 mg kg^{-1}	Nrf2/HO-1	Hong et al. (2014)
Pomolic acid	Renal interstitial fibrosis	BALB/c mice	0.4 mg kg^{-1}	↓Mothers against decapentaplegic homolog 3 (SMAD)-STAT	Park et al. (2018)
Pristimerin	Acute lung injury	Swiss albino mice	0.5 and 1 mg kg^{-1}	Antioxidant, anti-inflammatory, and anti-apoptotic pathways	Shaaban et al. (2018)
Pristimerin	Autoimmune hepatitis	Swiss albino mice	0.4 and 0.8 mg kg^{-1}	Nrf2/HO-1	Elagamy et al. (2018)
Pristimerin	Colon cancer	Female BALB/c mice	1 and 5 ppm	Akt/Forkhead box O3a (FOXO3a)	Park and Kim (2018)
Pristimerin	Rheumatoid arthritis	SD rats	0.4 and 0.8 mg kg^{-1}	Vascular endothelial growth factor receptor 2 (VEGFR2)	Deng et al. (2015)
Squalene	Ulcerative colitis	Female C57BL/6 mice	25 or 125 mg kg^{-1}	MAPK p38 and NF-κB	Sánchezfidalgo et al. (2015)
Ursolic acid	Lipid and glucose metabolism	C57BL/6J mice	50 or 200 mg kg^{-1}	PPAR-α	Jia et al. (2015)
Ursolic acid	Hepatic cirrhosis	C57/BL6 and AMPKα$2^{-/-}$ mice	50 mg kg^{-1}	Liver kinase B1 (LKB1)-AMPK	Yang et al. (2015b)
Ursolic acid	Hepatic inflammation	ICR mice	25 and 50 mg kg^{-1}	MAPK/NF-κB	Ma et al. (2014)
Ursolic acid	Chronic obstructive pulmonary disease	Wistar rats	10, 20, or 40 mg kg^{-1}	↓PERK ↑Nrf2	Lin et al. (2017)

(continued)

Table 4 (continued)

Compound	Disease	Animal model	Dose	Signaling pathway	References
Ursolic acid	Traumatic brain injury	ICR and Nrf2$^{-/-}$ mice	50, 100, or 150 mg kg^{-1}	Nrf2	Ding et al. (2017)
Ursolic acid	Ehrlich ascites carcinoma	Swiss female albino mice	25, 50, and 100 mg kg^{-1}	Mitochondrial-dependent pathway	Saraswati et al. (2013)
Ursolic acid	Non-alcoholic fatty liver disease	SD rats	0.125%, 0.25%, 0.5%	PPAR-α	Li et al. (2014a)
Ursolic acid	Allergic asthma	Female BALB/c mice	2 and 20 mg kg^{-1}	Th2-GATA-3 and IL-17-NF-kB	Kim et al. (2013b)
Ursolic acid	Spinal cord injury	Female C57BL/6J mice	100 and 200 mg kg^{-1}	MAPK, PI3K	Sahu et al. (2018)
Ursolic acid	Cerebral IRI	SD rats	10 and 20 mg kg^{-1}	High mobility group box 1 (HMGB1)/ TLR4/NFκB	Wang et al. (2018b)
Ursolic acid	Abdominal aortic aneurysm	ApoE$^{-/-}$ mice	100 mg kg^{-1}	STAT3, disintegrin, and metalloproteinase 17 (ADAM17)	Zhai et al. (2018)
Ursolic acid	Atherosclerosis	ApoE$^{-/-}$ mice	100 mg kg^{-1}	↓LOX-1 mediated by ROS/NF-κB	Li et al. (2018b)
Ursolic acid	Diabetic nephropathy	SD rats	35 mg kg^{-1}	Antioxidant and anti-inflammatory effects	Xu et al. (2018)
Ursolic acid	Liver fibrosis	SD rats	40 mg kg^{-1}	NOXs/ROS	Gan et al. (2018)

IRI making use of the left anterior descending coronary artery occlusion model of SD rats. Intraperitoneal administration of celastrol can attenuate histopathological changes, reduce myocardial injury, suppress myocardial apoptosis and autophagy, inhibit the oxidative stress and inflammatory response through activating the PI3K/Akt pathway, and suppress high mobility group box 1 protein (HMGB1) expression. Wang et al. (2018a) found that ursolic acid also participated in cerebral IRI via HMGB1/Toll-like receptor 4 (TLR4)/NFκB.

Diabetic nephropathy, a complication due to diabetes mellitus, is the second leading cause of end-stage renal disease and diagnosed by urinary albumin $\geq$30 mg g^{-1} Cr or estimated glomerular filtration rate (eGFR) of $\geq$30 mL/min/1.73 m^2. SD rats were subjected to streptozotocin 35 mg kg^{-1} intraperitoneally, including the model group, positive control group (metformin 150 mg kg^{-1} intragastrical), and treatment group (betulinic acid 20 or 40 mg kg^{-1} intragastrical). After execution, blood and kidney samples were collected; inflammatory cytokines, lipid peroxidation profile, blood glucose, and insulin level were measured; histopathological observation and Western blotting involving putative protein were carried out. Attenuating oxidative stress and inflammatory condition indicated that betulinic acid exhibited protection through the AMPK/NF-κB/Nrf2 signaling pathway (Xie et al. 2016). However, Wang et al. (2016b) thought that betulinic acid ameliorated diabetic nephropathy condition through enhancing the interaction between β-barrestin2 and IκBα and then affected the activation of NFκB. After the type 2 diabetes model was completed and successfully judged by the fasting blood glucose whether or not it is more than 16.7 mmol L^{-1}, the SD rats in the celastrol treatment group received dosage of 100, 200, and 500 μg kg^{-1} to explore its protective effect and its mechanism in diabetic mice. The results demonstrated that celastrol protected against diabetic nephropathy possibly related with TLR4/(myeloid differentiation factor 88) MyD88/NF-κB signaling (Han et al. 2016). Hou et al. (2017) administered 15 mg kg^{-1} glycyrrhizic acid intraperitoneally to db/db or db/dm mice for 8 weeks and illustrated the underlying mechanism related to AMPK/silent information regulator 1 (SIRT1)/PPAR-γ coactivator-1α (PGC-1α) signaling. Lee et al. (2015) studied the therapeutic effect and possible mechanism of oleanolic acid and *N*-acetylcysteine on diabetic nephropathy. When Long-Evans Tokushima Otsuka (LETO) rats were used as control, at the same time, Otsuka Long-Evans Tokushima Fatty (OLETF) rats were used as model, treatment groups were subjected to oleanolic acid or *N*-acetylcysteine for 20 weeks. Both the treated groups exhibited decreased urinary albumin/creatinine and triglyceride levels and increased superoxide dismutase and blood insulin secretion levels, which were mediated by oxidative stress and endoplasmic reticulum stress. The diabetic model induced by injecting streptozotocin 40 mg kg^{-1} intravenously was divided into three groups including model, treatment (ursolic acid 35 mg kg^{-1}), and positive control (telmisartan 12 mg kg^{-1}). At the last day, all SD rats were dissected, and their blood and kidney samples were preserved for the inquisitive purpose. Results showed that ursolic acid played a protective role in diabetic nephropathy due to its own antioxidation and anti-inflammation effects as indicated by Xu et al. (2018).

13.5 Benefits

Plenty of available preclinical evidences show that triterpenoids have multi-functional activities in terms of various malignant tumor and metabolic syndrome via intrinsic and extrinsic pathways. However, the failure rate of new drugs in the early clinical stage is as high as 90%. Even in the third phase, the failure rate is not less than 50%. Moreover, in view of the difference in characteristics of human and animal, the results of pharmacokinetics and pharmacodynamic need to be reassessed. In the early years, researchers made direct use of the extract containing active blended triterpenoids from plants to carry out clinical experiments. Limited contents act as chains for human experiments. In recent years, a batch of novel synthetic triterpenoids sprung up. According to the available data, clinical experiments can be classified into the following three phases: phase I includes the determination of dose-limiting toxicities, toxicity profile, pharmacokinetic analysis, and maximum tolerated dose; phase II is to measure pharmacodynamics relationship under the dose of maximum tolerance and determine appropriate dose; phase III is to observe the long-term safety and tolerability. Some clinical experiments of triterpenoids are summarized in Table 5.

M. charantia, known as bitter melon, is a herb plant used to heal diabetes and colic problem. Cortez-Navarrete et al. (2018) screened 24 eligible patients diagnosed with type 2 diabetes mellitus (T2DM), who were divided into two groups and given *M. charantia* extract (2 g day^{-1}) or placebo for 3 months. Furthermore, a randomized, double-blinded, and placebo-controlled clinical trial was conducted to investigate the therapeutic mechanism. They exhibited significant decrease in glycemic basic parameters such as weight, body mass index, fat percentage, waist circumference, and oral glucose tolerance test; conversely, the indexes about insulin were increased, which indicated that hyperglycemic action was related to insulin secretion instead of insulin sensitivity (Identifier: NCT02397447). Last year, Chung Shan Medical University as sponsor presided over a clinical experiment about exploring the efficacy of Greenyn *M. charantia* extracts on diabetes. Greenyn *M. charantia* extracts contain insulin receptor binding protein. Subjects were orally taking new drug or starch (600 mg day^{-1}) for 3 months, and then the fasting glucose, hemoglobin A1c, glucose tolerance test, and insulin sensitivity were measured; at the same time its safety was also evaluated (Identifier: NCT03151837).

Licorice, known as dried roots and rhizomes of *Glycyrrhiza glabra*, is used as a medicinal plant and cultivated throughout the world. Currently, researchers found that licorice extract possesses cytoprotective, hepaprotective, anti-inflammatory, antioxidant effects which aid in enhancing the activity of glycyrrhizic acid which has aroused interest in functional foods as sweetening additive. Ravanfar et al. (2016) selected 75 acute ischemic stroke (AIS) patients to implement human studies. All of them fell into three groups one placebo and two extract (450 mg day^{-1} and 900 mg day^{-1}) in which glycyrrhizic acid took up 7.85% and was lower than safety limitation (217 mg). The national institute of health stroke scale and modified rankin scale scores confirmed that licorice whole extract was safe, tolerable, and efficient for alleviating the adverse effects caused by AIS (Identifier: NCT02473458).

Table 5 Clinical experiments of triterpenoids

Compound	Model	Dosage	Results	Identifier	References
CDDO	Solid tumor	0.6–38.4 mg/m^2/h	MTD and DLT no determinate	NCT00352040	Speranza et al. (2012)
CDDO-Me	Solid tumor	5–1300 mg day^{-1}	MTD (900 mg day^{-1}), DLT (1300 mg day^{-1})	NCT00529438	Hong et al. (2008, 2012)
CDDO-Me	T2DM/CKD	25 and 75 mg	Kidney function ameliorate	NCT00811889	Pergola et al. (2011)
CDDO-Me	Healthy adults	20, 60, 80 mg	Safe and well tolerated	NCT01461161	Teuscher et al. (2014)
GA	Healthy adults	130 mg	Attenuate vascular smooth muscle vasodilatory function	NCT00759525	Sobieszczyk et al. (2010)
18β-GA	Chronic hemodialysis	500 mg	Lower serum potassium	NCT00384384	Farese et al. (2009)
Licorice extract	Acute ischemic stroke	450/900 mg day^{-1}	Improve neurologic	NCT02473458	Ravanfar et al. (2016)
Loquat extract	Healthy adults	500 mg day^{-1}	No differences in muscle mass, muscle strength, and physical performance	NCT02401113	Cho et al. (2016)
M. charantia extract	T2DM	2 g kg^{-1}	Modify the parameters of insulin secretion	NCT02397447	Cortez-Navarrete et al. (2018)
Omaveloxolone	Solid tumor	2.5 mg day^{-1} (12 cycle/28 days)	Safe and well tolerated	NCT02029729	Creelan et al. (2017)
Ursolic acid	Metabolic syndrome X	150 mg	Increase insulin sensitivity	NCT02337933	Ramírez-Rodríguez et al. (2017)

Sobieszczyk et al. (2010) selected 15 health subjects who received oral dosage of 130 mg once daily for 2 weeks, either GA or placebo, to verify whether decreased 11β-hydroxysteroid dehydrogenase (11β-HSD) activity caused by GA would affect vascular functions. They found that GA treatment significantly reduced serum potassium concentrations and plasma aldosterone concentrations by measuring and analyzing hemodynamic parameters. Thus, GA, as selective inhibitor of 11β-HSD, is effective for attenuating vascular smooth muscle vasodilatory function (Identifier: NCT00759525). Farese et al. (2009) randomly allocated 20 maintenance hemodialysis patients into two groups, who were given 18β-GA or dextrose 500 mg twice a day for 6 months. During the measurement, serum and hematology parameters were assessed. 18β-GA can reduce serum potassium concentration in chronic hemodialysis patients, which may be related to rectal and colonic loss (Identifier: NCT00384384).

Ramírez-Rodríguez et al. (2017) evaluated the effect of ursolic acid in metabolic syndrome X in 24 patients, who received 150 mg ursolic acid or calcined magnesia once a day before breakfast for 12 weeks. Ursolic acid administration can increase insulin sensitivity and modulate conventional index and leads to transient remission of metabolic syndrome. (Identifier: NCT02337933). Loquat extract used as dietary supplement was orally given to 54 sarcopenia patients (500 mg), which contained ursolic acid (50.94 mg), per day for 12 weeks. Cho et al. (2016) concluded that effect of Loquat extract exhibited no difference in muscle strength, muscle mass, and physical performance; however, it significantly influenced the right-handgrip strength of female subjects as indicated by the appendicular skeletal mass, quadriceps muscle power, hand grab power, and short physical performance battery (Identifier: NCT02401113).

In view of the slender efficacy of natural occurring triterpenoids, it is urgent to develop novel triterpenoids. To date, there exist CDDO and its ramification such as CDDO-Me, CDDO-Im, CDDO-EA, all of which belong to patent triterpenoid acids such as oleanolic acid, ursolic acid, and betulinic acid. Animal in vivo studies verified that synthetic triterpenoids almost were Nrf-2 activator, which mediated the anti-inflammation and antioxidative pathway. Many researchers paid significant attention to CDDO-Me. At present, there exist 30 clinical studies reporting registered use of CDDO-Me as drug, including chronic kidney disease (CKD), alprot syndrome, advanced solid tumors, lymphoid, diabetic nephropathy, and T2DM, even health subjects; nevertheless, less than 50% have been completed. Teuscher et al. (2014) assessed the safety, pharmacokinetics, and tolerance power of CDDO-Me with 20, 60, and 80 mg doses under the interference of food intake. It is safe and well tolerated without regard to meal times for healthy volunteers. Pergola et al. (2011) inspected long-term efficacy and dose effects by comparing eGFR with before or after CDDO-Me administration to patients diagnosed with T2DM and CKD. When three different dose groups were administered, i.e., 25, 50, and 75 mg, significant increase in eGFR values was observed through consecutive 52 weeks, indicating CDDO-Me is a promising candidate for treating CKD (Identifier: NCT00811889). Hong et al. (2008) reported the primary results about the pharmacokinetic parameters of CDDO-Me in 34 patients with advanced solid tumors or

lymphoid malignancies. The maximum tolerant dose (MTD) was 900 mg day^{-1}, whereas the dose-limiting toxicity (DLT) was 1,300 mg day^{-1} (Identifier: NCT00529438).

Creelan et al. (2017) selected 11 patients with confirmed melanoma or non-small cell lung cancer (NSCLC) to determine the safety and pharmacokinetics of omaveloxolone. Despite dose-limiting toxicities and maximum tolerated dose deficiency, it was well tolerated and safe in experimental dose (Identifier: NCT02029729). Since 2014, omaveloxolone or RTA 408 has been administered as drug, and 10 projects have been launched out of which seven were completed. The latest one is to assess the effects of food or dosage on omaveloxolone pharmacokinetics. They plan to recruit 32 healthy adult people to conduct three-phase studies by measuring pharmacokinetics parameters especially C_{max} and AUC (Identifier: NCT03664453).

13.6 Application in Food

The World Health Organization pointed out that less than 80% of human beings eat traditional medicines from herb plant extracts or active ingredients to meet their healthcare needs (Craig 1999). Triterpenoid-rich plants have wide varieties and are distributed extensively worldwide. Through the ages, people made use of these plants to prevent common chronic ailments such as fever, cough, hypertension, diabetes, and inflammation. These plants are safe enough to be used as dietary supplements. In view of the abovementioned discussion, mounting attention has been paid in nutraceutical and pharmaceutical applications of triterpenoids in the recent years. Noteworthy, the toxicity of pure compounds needs to be verified by animal and human studies.

Glycyrrhizic acid is mainly obtained from three licorice species, namely, *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* Fisch., and *Glycyrrhiza inflata* Bat., and it is the most dominant active constituent of licorice root extract in the proportion of 10–25%. Traditionally, licorice was found to be useful for persons with symptoms of peptic ulcers, malaria, abdominal pain, asthma, pharyngitis, insomnia, and infections. Correspondingly, glycyrrhizic acid exhibited antigenotoxic, antitussive, antiviral, anti-inflammatory, and gastroprotective properties. Owing to high-sweetness (170-fold than sucrose), low-calorie, flavor-enhancing, and salt-softening functional food properties, glycyrrhizic acid is applied as sweetening and flavoring agent in tobacco flavors, beverages, cocoa, and confectionery products. Furthermore, glycyrrhizic acid can be applied in beer and ale products in light of good foamability and high sweetness which can neutralize bitter taste. However, glycyrrhizic acid may produce undesirable brownish color and lessen sweetness in acidic solutions (Isbrucker and Burdock 2006). GA is formed by hydrolysis of glycyrrhizic acid by intestinal bacteria by virtue of glucuronidase, and it has strong sweetness (941 times than sucrose); thus it can be used in same fields mentioned above (Hayashi and Sudo 2009).

Siraitia grosvenorii (Swingle) is a familiar fruit named "luo han guo" in China and is used to alleviate sore throats, cough, and minor stomach and intestinal troubles. Its extract can be consumed as a sugar substitute for diabetic patients due to the presence of minimal caloric cucurbitane-type glycosides containing primarily mogroside IV, V, and VI. The extract has been reported to be 300 times sweeter than sucrose. Thus, *S. grosvenorii* extract can be utilized in soft drinks, cereals, and nutritional and energy shakes in the form of sweetener and flavor enhancer (Pawar et al. 2013).

M. charantia is a known vegetable named as bitter melon and has the functions of clearing away heat and detoxification, nourishing and rejuvenating skin, decreasing blood sugar, nourishing the blood and liver, and anti-diabetes, or other related complications. Despite 288 compounds reported in *M. charantia* from different tissue extracts, cucurbitane-type triterpenoids were recognized as potential food supplements to prevent T2DM. To date, *M. charantia* is found in health drink, bitter gourd tea, preserved fruit, candied fruit, and kimchi (Nagarani et al. 2014).

Centella asiatica is the most ubiquitous, among which 50 *Centella* species belong to Apiaceae or Umbelliferae. It is popular in Asian countries as herb medicine to treat insanity, asthma, ulceration, wound healing, headache, body ache, and eczema. Its extract takes effect mainly because of its component, centellosides. In fact, centellosides are a mixture of various triterpenoids including asiatic acid, madecassic acid, asiaticoside, and madecassoside. Similarly, centellosides have memory-enhancing, antidiabetic, neuroprotective, anti-inflammatory, antioxidant, wound healing, and anticancer properties. In view of these properties, *C. asiatica* has been utilized to develop tonic drinks for general health and blood circulation (Hashim 2013).

Oleanolic acid has been isolated from more than 1600 plant species, and a range of benefits has been reported. Oleanolic acid takes up approximately 3% in the leaf of dried *Olea europaea*. Incorporating oleanolic acid into basic products can improve the nutritive value of the patent products, which finally turn into functional foods for healthcare consideration. Guinda et al. (2004) explored the process of prepared supplement oil with oleanolic acid and found that it was better to use solid oleanoic acid in concentration less than 400 ppm; conversely dissolved option can also be used.

Squalene is an acyclic triterpenoid directly related to cholesterol synthesis and exhibits strong antioxidation, antiaging, cardioprotective, and photoprotect capabilities. It has been found in shark liver oil (<40%), amaranth oil (<10%), olive oil (<1%), various oils, and human sebum. Squalene is popular in cosmetics and dietary supplement and also used as an additive in animal feed (Kumar et al. 2017).

It is estimated that ginsenosides are not less than 150 in number and form a large group of triterpenoid saponins isolated from diverse tissues of *Panax* species. According to unique nature of aglycone moieties, protopanaxadiol and protopanaxatriol are the chief bioactive ingredients including Rb1, Rb2, Rb3, Re, Rh1, Rh2, Rh3, Rf, Rg1, Rg2, Rg3, Rg5, and others. Ginsenosides are effective in treating liver diseases, eyesight troubles, and cardiovascular and gastrointestinal problems. Previously, native Brazilians deemed "Brazilian ginseng" to be "for all

things," in consideration of its uses as a tonic, an aphrodisiac, and a remedy for diabetes, ulcers, cancer, and others. In the United States, ginseng is used not only as a dietary supplement but also in a series of products such as milk, tea, crunchy white chocolate, dark chocolate, and candy (Chung et al. 2011).

Western medicine produces toxicity and resistance through long-time administration. Therefore, progressively more attention should be paid to functional foods with nontoxic and health-promoting characteristics, which mediate basic foods and medicines. To date, commercial products mainly involve tea, beverages, olive oil, and confectionery industries. Moreover, dietary supplements make a good measure, which incorporate bioactive triterpenoids as ingredients.

13.7 Safety: Toxicity and Side Effects

The safety of triterpenoids either as extracts or pure compounds is very crucial for the development of clinical experiments and healthcare products. In the duration of several decades, researchers carried out various toxicity tests which were also related to the study of activities, sometimes. Toxicity tests generally cover acute, subacute, chronic, genetic, and other special toxicities. Table 6 summarizes the toxicity of triterpenoids.

Bamboo shavings are dried intermediate species of stems and are used to treat hot cough, biliary sputum, convulsions, diarrhea, feeling upset, insomnia, excessive thirst, stroke, fascination, chest diaphragm inflammation, stomach heat, vomiting, pregnancy resistance, and fetal restlessness. Bamboo shavings extract (BSE) contains polyphenol, flavonoids, and friedelane- or lupane-type triterpenoids. Zhang et al. (2004) handled safety evaluation for acute toxicity and mutagenicity and 30-day feeding test from three aspects. SD rats and Kunming strain mice were administered with 1, 2.15, 4.64, and 10 g kg^{-1} BSE for 14 days, and then the acute toxicity was judged by calculating MTD and observing general status, toxic symptom, and mortality in rats. Mutagenic toxicity was evaluated by carrying out Ames test, mice micronucleus test, and sperm abnormality test using Kunming strain mice. Then, SD male and post-weaning female rats were divided into four groups, and dosages of 0, 0.21, 0.42, and 0.83 g kg^{-1} BSE, respectively, were administered. Further, the changes of the liver, kidneys, spleen, testes, and ovaries were observed by paraffin section, hematoxylin-eosin staining, and photoscope detection. BSE did not show any acute toxicity symptoms, so the MTD of BSE >10 g kg^{-1}. All the results of mutagenicity tests were negative. No obvious toxicity, pathological changes, and adverse effects were exhibited in the 30-day feeding test.

Isbrucker and Burdock (2006) reported the safety profiles of licorice extract including its main bioactive compounds systematically. In simple terms, glycyrrhizic acid was found to be without expressed acute toxicity below 4 g kg^{-1}. Based on the 30-day feeding test, no-observed effect levels were in the range of 15–229 mg kg^{-1}. When the dosage is below 4–5 g kg^{-1}, glycyrrhizic acid leads to mutagenic effects in offspring.

Table 6 Toxicity of triterpenoids

Compound	Model	Toxicity	References
Birch triterpenoid extract	Acute-Swiss mice	No symptoms of mortality or toxicity occurred within 15 days of taking <2 g kg^{-1}	Majeed et al. (2014)
BSE	Acute-SD rats and Kunming strain mice	No symptoms of mortality or toxicity occurred within 14 days of taking <10 g kg^{-1}	Zhang et al. (2004)
BSE	Mutagenicity-Kunming strain mice	All of the results of Ames test, mice micronucleus test, and sperm abnormality test are negative	Zhang et al. (2004)
BSE	30-day feeding test	No difference in hematology, clinical chemistry, and histopathological results	Zhang et al. (2004)
Ganoderma lucidum total triterpenes	Acute-Swiss albino mice	No symptoms of mortality or toxicity occurred within 14 days of taking <5 g kg^{-1}	Smina et al. (2011)
Ganoderma lucidum total triterpenes	Subacute-Swiss albino mice	No changes in parameters of hematological and biochemical within 30 days of giving <0.5 g kg^{-1}	Smina et al. (2011)
Hydroalcoholic solution of triterpenes	Mutagenicity TA98, TA1535, and YG1024	No mutagenicity	Lupi et al. (2009)
Methyl 3-octanoyloxyiminoolean-12-en-28-oate	Acute-Wistar rats and Swiss mice	No symptoms of mortality or toxicity occurred within 14 days of taking 2 g kg^{-1}	Bednarczyk-Cwynar et al. (2012)
Oleanolic/ursolic acid	Acute-BALB/c albino mice	No symptoms of mortality or toxicity occurred within 72 h of taking 2 g kg^{-1}	Resende et al. (2006)

Lupi et al. (2009) isolated hydroalcoholic solution of triterpenes (8.323 mg L^{-1}) from *Boswellia sacra* resin, *Commiphora myrrha* resin, and *Hyssopus decumbens* essential oil by electromagnetic field extraction. S9 was selected as metabolic activator to carry out Ames tests, which showed the absence of mutagenicity in *Salmonella typhimurium* (TA98, TA1535, and YG1024).

Triterpenoid-rich plant extracts have low triterpenoid content, so they are non-toxic when the content is below 1 g kg^{-1}. In contrast, owing to the difference of physical condition in human or the out of range dosage, there are many side effects in clinical experiments, which even led to the termination of studies. Therefore, Table 7 lists some terminated clinical experiments or some exceptions in completed clinical experiments.

Table 7 Side effects of triterpenoids

Compound	Model	Side effects	References
CDDO	Solid tumor	Pulmonary embolism	Speranza et al. (2012)
CDDO-Me	CKD/T2DM	Nonfatal myocardial infarction, nonfatal stroke, hospitalization for heart failure, or death from cardiovascular causes	De zeeuw et al. (2013)
CDDO-Me	CKD/T2DM	Heart failure	Chin et al. (2014)
CDDO-Me	CKD/T2DM	No adverse effects	Pergola et al. (2011)
CDDO-Me	Health adult	Abdominal pain, diarrhea, urinary tract infection, headache	Teuscher et al. (2014)
GA	Chronic hemodialysis	Diarrhea (minority)	Farese et al. (2009)
GA	Health adults	No adverse effects	Sobieszczyk et al. (2010)
Licorice root	Acute ischemic stroke	No adverse effects	Ravanfar et al. (2016)
M. charantia	T2DM	Headache and dizziness, nausea, vomiting, constipation	Cortez-Navarrete et al. (2018)

Phase 3 occurrence of renal events (BEACON) trial was terminated in the condition of increased mortality related to fluid retention. Chin et al. (2014) provided a hypothesis that CDDO-Me could lead to acute sodium retention and increase blood pressure in patients with T2DM and stage 4 CKD, via modulating endothelin pathway. Given the same clinical phenotype, this mechanism can be extrapolated to the reason why the BEACON experiment failed (Identifier: NCT01549769; NCT01351675).

13.8 Marketed Products

In light of the potential bioactive and well-tolerated characterization of triterpenoids, increasing number of businessmen put their eyes on the development involved in pharmaceutical, food, healthcare products, and other industries. In general, addition of triterpenoids in the form of direct raw materials or as dietary supplements to low nutritional commodities, in order to endow additional value to patent products, results in the increase in the efficiency in the treatment of diabetes, stomach problems, hepatitis, coughing, and other illnesses. Traditional medicinal plants have a long history, and their efficacy and safety can be guaranteed. At the same

time, there have been many studies on the main active ingredients in medicinal plants in the past 100 years. For example, *Ganoderma lucidum* (lingzhi) can prevent bronchitis, hepatitis, hypertension, arthritis, and nephritis (Ye et al. 2018). *Antrodia cinnamomea* (Niuzhangzhi) plays a curative role in abdominal pain, itchy skin, intoxication, diarrhea, and inflammation (Qiao et al. 2015; Huang et al. 2018). *Schisandra chinensis* (Wuweizi) can defend from cough, fatigue, rheumatism, amnesia, contusions, insomnia, and arthritis (Qiu et al. 2018; Szopa et al. 2017). *Glycyrrhiza uralensis* (Gancao) can be utilized for the treatment of asthma, chronic gastritis, bladder infection, and atopic dermatitis (Schmid et al. 2018). *Poria cocos* (Fuling) is effective for insomnia, dysfunction, diarrhea, and urinary problems. In recent years, literature studies reported that abovementioned medicines contain ganoderic acid, eburicoic acid, schinenlactone, glycyrrhizic acid, and pachymic acid, respectively.

For dietary supplements, their functions mainly focus on enhancing immunity, improving hypoxia tolerance, relieving physical fatigue, improving assisted memory, reducing blood fat, regulating blood sugar level, meliorating sleep, and others. In China, there exist more than thousands of nutraceutical health products which are actually a mixture of traditional herbs or their extracts. Their categories are widely found in tea, wine, drinks, oral solution, etc.

For cosmetics, oleanolic acid, ursolic acid, glycyrrhizic acid, ginsenosides, asiaticoside, and others have been registered in China Food and Drug Administration, among which the emblematic one is *Centella* extracts. *C. asiatica* extract can tightly connect the epidermis to the dermis, promote the formation of collagen in the dermis layer, and regenerate fibrin, thereby relaxing the skin, reducing the tension of the skin, and making the skin firm, smooth, and elastic. Moreover, *C. asiatica* extract can repair damaged skin tissue, treat skin ulcers, calm skin, and relieve sensitive symptoms. At present, many international makeup brands use the *C. asiatica* extract in their products as a promotional hotspot. In America, France, Italy, Korea, and China, this extract has been used in mask, water, cream, emulsion, recovery skin-salve, sunscreen emulsion with anti-wrinkle effect, tightening, whitening, freckle, sunscreen, repair, and other functions. In Korea, oleanolic acid and salvianolic acid have been employed in anti-wrinkle or skin-whitening emulsion cosmetics.

In the field of pharmaceuticals, the ancient prescriptions used in all the countries are derived from the mixture of many traditional herbs. With the in-depth study of plant extracts, a large number of pure products have been made into decoctions, tablets, capsules, and granules to assist in the treatment of some diseases. For instance, WS® 1442 isolated from hawthorn leaves with flowers was found to consist of triterpenoids, flavonoids, phenol carboxylic acids, and oligomeric procyanidins. As nutraceuticals, it is used to treat early stages of congestive heart failure in many European countries. In vitro and in vivo tests have shown that it is safe, is well tolerable, and has great potential to treat heart failure and improve exercise capacity (Holubarsch et al. 2008).

Table 8 summarizes the representative marketed products and their indications from triterpenoid-rich plant or synthesis of novel compounds.

Table 8 Marketed products of triterpenoids

Products	Constituent	Indications
Asiaticosides	Centella	Trauma, surgical trauma, burns, keloids, and scleroderma
Asiaticoside ointment	Centella	Wound, ulceration, scorching, adhesion, cicatrix, scleroderma
Compound centella tablet	Centella	Invigorate blood circulation, remove blood stasis, and relieve pain. Used for falls and injuries, limb pain
Compound glycyrrhizin tablets	Glycyrrhizic acid	Chronic liver disease, improving liver function abnormalities, eczema, dermatitis, alopecia areata
Compound glycyrrhizin injection	Glycyrrhizic acid	Chronic liver disease, improving liver function abnormalities, eczema, dermatitis, urticaria
Extractum glycyrrhizae liquidum	Licorice	Bronchitis, pharyngitis, bronchial asthma, chronic adrenal insufficiency
Renshen guipi wan	Ginseng	Deficiency of qi and blood, palpitation, insomnia, lack of energy and diet, and yellow complexion
Renshen jianpi wan	Ginseng	Using for the diet caused by spleen and stomach weakness, wan stuffy and noisy, nausea and vomiting, abdominal pain and loose stools, not thinking about diet, weakness and tiredness.
Renshen yangrong wan	Ginseng	Using for deficiency of heart and spleen, deficiency of qi and blood, thin body and weak spirit, little food and loose stool, weakness after disease.
Renshen zaizao wan	Ginseng	Invigorate qi and blood, dispel wind and phlegm, activate blood circulation
Ganoderma lucidum nanogels	*Ganoderma lucidum*	Frostbite
Sanqi tablets	Panax nonginsheng	Using to dissipate stasis, stop bleeding, reduce swelling and relieve pain
Panax notoginseng hemostatic tablet	Panax nonginsheng	Stasis to stop bleeding, detumescence, pain
Astragalus granule	*Astragalus*	Shortness of breath, palpitation, spontaneous sweating
Astragalus jianwei ointment	*Astragalus*	Stomachache caused by deficiency of the spleen and stomach
Ganoderma capsule	*Ganoderma*	Insomnia, forgetfulness, physical weakness, neurasthenia
Fufang Lingzhi granules	*Ganoderma*	Acute infectious jaundice hepatitis, chronic hepatitis
G. lucidum spore powder capsule	*Ganoderma lucidum*	Deficiency of the heart and spleen, weakness after disease, adjuvant therapy for tumor patients
G. lucidum spores softgel	*Ganoderma*	Boost immunity
Guizhi Fuling capsule	*Poria cocos*	Invigorate blood circulation, remove blood stasis, and eliminate disease
Jingpai yunjiu	*Poria cocos*	Remove chloasma and enhance immunity
Oleanolic acid tablets	Oleanolic acid	Adjuvant therapy for acute and chronic hepatitis

13.9 Patents

Many patents on triterpenoid compounds are available, which can roughly be classified into five categories, namely, extraction of compounds and their separation methods, synthesis of compounds and their synthesis methods, methods for improving the drug properties of compounds, research and development of products, and others. Table 9 lists some patents based on these aspects.

The active ingredients and their contents in different plants vary significantly. The solvent, temperature, ratio, and other process parameters affect the extraction efficiency. Therefore, many patents are related to the process optimization of extracting total triterpenes from plants, such as *Dracocephalum moldavica*, glossy privet fruit, scandent schefflera, *Chaenomeles* fruit, and spina gleditsiae. With the development of technology, people continue to simplify the process or increase yield from organic solvent extraction to subcritical water extraction and then supercritical extraction. These patents on synthesis mainly include a method for making synthetic triterpenoids used in the treatment and prevention of various diseases, such as stimulating bone and cartilage growth and improving chronic/acute kidney/liver disease, neurodegenerative diseases, cardiovascular disease, and various types of cancers. These patents on methods for improving pharmacokinetics properties accord with the aforementioned information in Sect. 3.1 including SMDDS, reducing particle diameter and binding with hydrophilic substances CDs, polygalacturonic acid, and others. These patents on activity study keep pace with the discussion in Sect. 4. These studies are related to either mixtures or semi-pure compounds, and a large number of animal studies were carried out to verify their efficacy in cancer, liver disease, T2DM, CKD, and so on. These patents on research and development of products disclose many formulas about products containing triterpenoids or their plant origin which are widely used in nutraceuticals, pharmaceuticals, cosmetics, and even industrial products.

13.10 Perspectives

Triterpenoids are important natural secondary metabolic products derived from crucial medicinal plants such as *Panax ginseng*, *Glycyrrhiza uralensis*, Radix Bupleuri, etc. Overwhelming evidence has shown that triterpenoids possess anti-inflammation, anti-obesity, antidiabetic, and anticancer efficacy through plurality of common signaling pathway in animal experiments. However, there are several challenges to overcome. First, inferior bioavailability caused by weak hydrophilia and poor gastrointestinal absorption limit the practical application of triterpenoids. Although there are abundant ways to enhance their bioavailability, no method has been found that is propitious to industrial production or successful in clinical trials. Second, their commercialization includes low production levels and cost-effective purification from the complex mixtures present in their natural hosts. Limited production leads to insufficient quantity and purity to carry out further animal and human experiments. Third, with respect to therapeutic mechanism, a lot more

Table 9 Patents on triterpenoids

Category	Main content	References
Extraction of compounds and methods	Separation of high-purity ursolic acid from loquat leaves by subcritical water extraction technology, which can be used as a functional factor in pharmaceuticals, healthcare products, and cosmetics	Chen et al. (2012a)
	The triterpenoids were extracted from apple pomace by organic solvent extraction method. The process was optimized and then made suitable for industrial production. At the same time, the composition analysis and activity study were also carried out. The results showed that the compound mainly contained ursolic acid and oleanolic acid, and it could protect against carbon tetrachloride-induced acute liver injury models and could be used in the development of health supplements	Ren and Zhang (2015)
	Direct separation and purification of triterpenoids and polysaccharides from *Antrodia camphorata* via supercritical fluid technology. This method is simple in process, cost-effective, solvent-free, safe, and environmentally friendly	Chuang et al. (2016)
	Using edible oil and fat as entrainer, supercritical carbon dioxide extraction technology was used to prepare high-efficiency anti-tumor *Ganoderma lucidum* extract, which is rich in variety, high in total content, stable in traits, safe to eat, and can be used in healthcare products	Xu et al. (2015)
Synthetic compounds and methods	Betulin ramifications are useful to inhibit various cancers (e.g., ovarian cancer, breast cancer, colorectal cancer, cervical cancer, and glioblastomas)	Xu et al. (2008)
	New compounds and methods are used to treat neurodegenerative diseases, psychiatric disorders, chronic pain, spinal cord injuries, and other injuries, which overcome current technical limitations	Sporn et al. (2009)
	Certain triterpenoids can treat bone/cartilage diseases via inducing gene expression and differentiation of stem or progenitor cells	Sporn et al. (2014)
	CDDO and its synthetic derivatizations can come into play in treating and preventing renal/kidney disease, endothelial dysfunction, fatty liver disease, insulin resistance, diabetes, and cardiovascular disease	Sporn et al. 2018)
Methods for improving pharmacokinetics properties	The application of the SMDDS to the preparation of the *Ganoderma lucidum* triterpenoid extract can improve the dissolution rate of the drug, so that it can be	Chen et al. (2009)

(continued)

Table 9 (continued)

Category	Main content	References
	rapidly absorbed by the gastrointestinal tract, improve the bioavailability, and completely exert the drug effect in the body	
	Lupane-, oleanane-, and lanostane-type triterpenes isolated from medical natural products were made into supramolecular nanoparticles which could be easily absorbed through the gastrointestinal tract, and then the bioavailability and intestinal permeability advanced significantly	Pabst et al. (2018)
	Oleanolic acid combines with polygalacturonic acid to form nano-sized micelles through esterification reaction. By drug targeted transportation, the bioavailability of oleanolic acid can be enhanced, insulin resistance can be better inhibited, and insulin sensitivity can be improved	Guan and Li (2017)
	The non-crystalline, glassy solid form of CDDO-Me displays an improved bioavailability over the non-hydrous crystalline form	Walling et al. (2009)
Activity study	Cucurbitane tetracyclic triterpenoids mainly include mogroside groups purified by macroporous resin separation and high pressure reversed-phase preparative chromatography. These compounds can inhibit pulmonary fibrosis via reducing accumulation volume of collagen in alveolar epithelial–mesenchymal and inflammation; thus these compounds are promising as a drug or healthcare product for pulmonary fibrosis	Xie et al. (2017)
	According to changes in relevant clinical indexes, it can be judged that celastrol and derivatives can prevent obesity	Mazitschek and Ozcan (2017)
	The triterpenoid compound extracted from the *Poria cocos* peel can reduce the degree of kidney disease, urine protein content, and creatinine and urea nitrogen and plays a therapeutic role in kidney disease	Yan et al. (2017)
	Maslinic acid can alter the mechanism of cell excitability and can aid in the treatment of central nervous system diseases such as depression, Parkinson's, and spinal injuries	Li et al. (2017a)

Research and development of products	Bitter substances (3–9%) from *Ganoderma lucidum* are added to flour to make vermicelli with antiallergy, antiandrogen, and antihypertension effects	Lee (2003)
	A mixture of triterpenoids, polyphenols, and similar compounds is extracted from five plants such as *Centella asiatica* using an ethanol solution, and animal experiments confirmed that the mixture is helpful for improving oral diseases and can be added to toothpaste, mouthwash, and chewing gum preparations	Li (2015)
	The special treatment of broken *Ganoderma lucidum* spore powder can increase the content of its functional active ingredients, help to enhance immunity, prevent radiation damage, and better play its role as a healthcare product	Li et al. (2018a)
	Ganoderma lucidum superfine powder, *Ganoderma lucidum* extract powder, and *Ganoderma lucidum* broken spore powder are blended into new *Ganoderma lucidum* powder in an appropriate ratio, which can increase anti-liver tumor activity	Liu et al. (2017a)

systematic explorations are still needed. Finally, the research lacks extremely in evaluation of pharmacokinetics and pharmacodynamics of triterpenoids.

13.11 Cross-References

- ▶ Alkaloids in Diet
- ▶ Anthocyanins in Food
- ▶ Antioxidants in Diets and Food
- ▶ Biflavonoids and Oligomeric Flavonoids from Food
- ▶ Dietary Diterpenoids
- ▶ Dietary Ellagitannins
- ▶ Dietary Flavonols and O-Glycosides
- ▶ Dietary Monoterpenoids
- ▶ Flavonoid C-Glycosides in Diets
- ▶ Gallotannins in Food
- ▶ Ginsenosides in Diets
- ▶ Introduction of Phytonutrients
- ▶ Saponins in Food
- ▶ Sesquiterpenes in Cereals and Spices
- ▶ Sesquiterpenes in Fresh Food
- ▶ Tea Catechins

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Sesquiterpenes in Fresh Food

14

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Contents

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Abstract

Sesquiterpenes are secondary metabolites that have interesting health-promoting functions, including anti-inflammatory, cardiovascular, gastro-protective, and anticancer properties. The potential of these compounds in human health will be further discussed throughout this chapter, being incorporated as part of a balanced diet and as pharmaceutical agents. Several foods consumed daily are a rich source of these compounds such as fruits, vegetables, algae, edible medicinal plants, and mushrooms. An overview of the bioactive constituents found in these aliments, metabolism routes and bioavailability, benefits for human health, safety upon ingestion, and market products will be described. Moreover, a deep understanding of the chemical constituents and biological activity observed will be detailed (structure-activity relationship studies). On the other hand, some of the peculiar aspects of these compounds (for instance, sesquiterpene lactones) is the bitterness that they confer to foods such as chicory, lettuce, or dandelion. This bitter taste is a direct consequence to their role as antifeedants, and this characteristic might help to ascertain the appropriate daily doses of sesquiterpenes. Little is known about the intake of sesquiterpenes in our diet. This chapter provides an understanding of the role of sesquiterpenes in health and human diet and the importance of increase their comsumption.

Keywords

Sesquiterpenes · Fruits · Vegetables · Algae · Mushrooms · Functional food · Edible medicinal plants

Abbreviation

A-549	Adenocarcinomic human alveolar basal epithelial cells
ATP	Adenosine triphosphate
B16F10	Murine melanoma cell line
BEL-7402	Human hepatoma cell line
BGC-823	Human gastric cancer cell line
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
D-GaIN/LPS	Lipopolysaccharide/D-galactosamine
DMAPP	Dimethylallyl diphosphate
EGb	Standardized *Ginkgo biloba* leaf extract
FPP	Farnesyl pyrophosphate
FPS	Farnesyl pyrophosphate synthase
GA3P	Glyceraldehyde 3-phosphate
GABA	γ-Aminobutyric acid
GOT	Glutamate-oxaloacetate aminotransferase
GPT	Glutamate-pyruvate aminotransferase
H_2O	Dihydrogen oxide
HCT-8	Human ileocecal adenocarcinoma cells
HeLa	Human cervical cancer cell line (Henrietta Lacks)
HSV-1	*Herpes simplex* virus type 1
I.929	Mouse fibroblast cell line
IC_{50}	Half maximal inhibitory concentration
ID_{50}	Half infectious doses
iNOS	Inducible nitric oxide synthase
IPP	Isopentenyl diphosphate
LD_{50}	Half lethal dose
LPS	Lipopolysaccharide
MDM2	Mouse double minute 2
MEP	Methylerythritol phosphate
MIC	Minimum inhibitory concentration
MVA	Mevalonic acid
NF-kB	Nuclear factor-kB
NGS	Next-generation sequencing
NLT	Not less than
NMT	Not more than
NO	Nitric oxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
OVCAR-3	Human ovarian carcinoma cell line

PAs	Pyrrolizidine alkaloids
PGE2	Prostaglandin E2
RAW	Monocyte/macrophage-like cells, originating from Abelson leukemia virus transformed cell line derived from BALB/c (albino, laboratory-bred strain) mice
RCR	Respiratory control ratio
RD	Raynaud's disease
SERCA	Sarco-/endoplasmic reticulum calcium
SK-N-SH	Neuroblastoma cell line
SLs	Sesquiterpene lactones
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor alpha
TPA	Tetradecanoylphorbol-13-acetate
TPS	Terpene synthase
USA	United States of America
UV	Ultraviolet
UVB	Ultraviolet B radiation
W	Watts

14.1 Introduction

14.1.1 Fruits

Fruits constitute an important part of the human diet. Fruits not only provide vitamins and other nutrients needed for life, but they also maintain health and prevent disease, including cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, and age-related deterioration. Given the nutritional benefits, fruit consumption has increased over the years. Although fresh or frozen fruits are more common, processed products account for a high percentage of worldwide production as juices and derivatives like alcoholic beverages (cider, wine, and spirits) and baby food (Liu 2003). A wide variety of phytochemicals can be found in fruits, and these include phenols, flavonoids, saponins, and acetogenins, among others. However, sesquiterpenes represent a significant fraction, especially in essential oils obtained from fruits. The most commonly consumed fruits in the world that are rich in sesquiterpenes are described below (Fig. 1).

14.1.1.1 Apple

A large number of edible apple varieties or *Malus domestica* spp. are cultivated around the world. Direct consumption and fabrication of cider and other derivative foods are the main destinations for apple crops. The composition of apples changes between the different varieties, and there are also small changes in phytochemicals during the maturation and ripening of the fruit. In contrast to other species, storage has additional effects on the chemical composition of apples. The most abundant

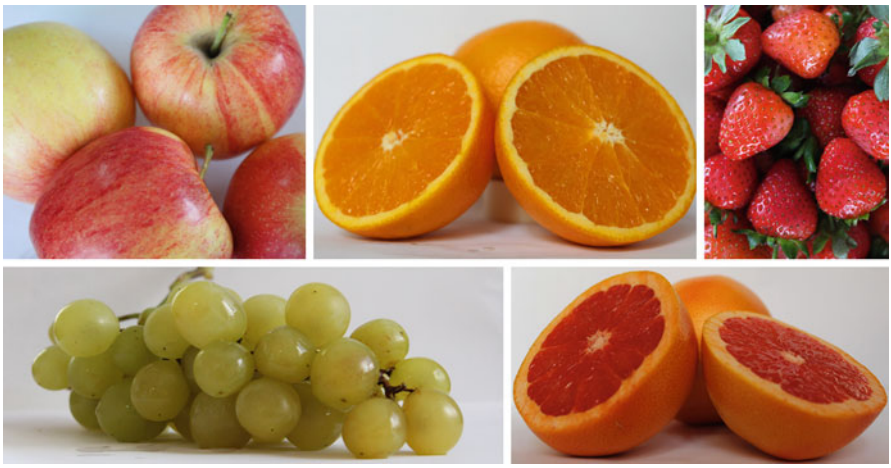


Fig. 1 Fruits that are rich in sesquiterpenes

sesquiterpene in the main varieties of apples is α-farnesene (Fig. 4; Ferreria et al. 2009; Rapparini et al. 2001).

14.1.1.2 Orange

Oranges are the fruit of the *Citrus x sinensis* species, a hybrid between *Citrus maxima* or grapefruit and *Citrus reticulata* or mandarin. Alkaloids, tannins, saponins, phenols, and flavonoids are described as phytochemicals in this fruit (Okwu and Emenike 2006), and several types of bioactivity have been associated with these fruits, e.g., antioxidant and anticancer effects. The most abundant sesquiterpenes found in several varieties of oranges are α-copaene, δ-cadinene, valencene, α-ylangene, β-caryophyllene, and β-elemene. In this chapter the first four compounds will be discussed, whereas β-caryophyllene and β-elemene will be described in the next chapter, although a brief introduction is provided here. β-Caryophyllene is found in grapefruit and grapes, and it is a primary component of spices. It is found in oregano, cinnamon, cloves, and rosemary and in herbs such as hops (common lupulus) or lavender and even in marijuana. On the other hand, β-elemene is an extended sesquiterpene that is found in a wide variety of plants.

14.1.1.3 Strawberry

Strawberries are a characteristic fruit from plants of the genus *Fragaria x ananassa*. A large number of health benefits have been reported for strawberries due to the presence of a wide variety of nutrients and the main phytochemicals, namely, polyphenols. It is worth noting that the sesquiterpenoid nerolidol is found within the characteristic volatile compounds.

14.1.1.4 Grapes

Grapes belong to *Vitis vinifera*, which is an extensively studied species, and they contain several sesquiterpenes, including β-elemene, β-bisabolene, β-caryophyllene, germacrene D, β-farnesene, α-humulene, farnesol, and α-bisabolol. Only farnesol will be discussed in this chapter because the other sesquiterpenes mentioned above are also present in other food types. Accordingly, these other sesquiterpenes will be described in more detail in the next chapter. For example, β-bisabolene is present in wild carrots, spices such as anise and oregano, and some herbs like Roman chamomile. The sesquiterpene α-humulene, also found in cannabis and in other aromatic herbs, is the main component in hops, and it is also found in beer. Moreover, the main component in chamomile is α-bisabolol, which is a widely used sesquiterpene due its protective effect on skin.

14.1.1.5 Grapefruit

The grapefruit, or *Citrus x paradisi*, is appreciated due to its high content in vitamin C, fiber, and lycopene. Moreover, several sesquiterpenes have been described in essential oils of this fruit, including α-copaene, α-humulene, β-caryophyllene, germacrene D, nerolidol, β-cubebene, and nootkatone.

14.1.2 Vegetables

Vegetables constitute a rich source of vitamins, essential minerals, fiber, and phytochemicals, and they are widely recommended for their health-promoting properties. In this work, the different sesquiterpenes found in vegetables will be discussed along with the potential of these compounds and their importance as part of a balanced, healthy diet (Chadwick et al. 2013).

14.1.2.1 *Curcuma zedoaria* (Christm.) Roscoe

Curcuma zedoaria (Zingiberaceae plant), commonly known as zedoary, has been cultivated as a vegetable or spice in South and Southeast Asian countries. The rhizomes of this plant have been widely used in Chinese and Japanese traditional medicine, and they are prescribed for stomachic complaints, as an emmenagogue and for the treatment of "Oketsu" syndrome (blood stasis or stagnant syndrome). This plant constitutes a rich source of sesquiterpenes, and it is worth highlighting carabrane-type (such as curcumenolactones A, B, and C and curcumenone), guaiane-type (4-epicurcumenol and alismoxide), and germacrane-type (glechomanolide and dehydrocurdione) sesquiterpenes, among others (Morikawa 2007).

14.1.2.2 *Cichorium intybus* L.

Chicory (*Cichorium intybus L.*) is an important agricultural crop in Northern Europe, and it is widespread in Europe, North Africa, and parts of Asia. The roots and leaves of this plant have several uses, for instance, the roots are cooked as a vegetable or roasted as a coffee surrogate, and they are an excellent source of inulin and fructose; the leaves are consumed as a vegetable and are used as livestock forage, especially

for ruminants; and the sprouts are consumed raw or as admixtures to salads (Barry 1998; Wang and Cui 2011). In northern France, chicory root is grown especially for use as dried or roasted products and in beverages. Once harvested, the root is sliced and dried to produce an intermediate raw material called green slices. The slices are either ground to obtain flour or roasted and then crushed to give grains. The grains can be marketed directly or extracted with hot water to obtain a concentrated liquid or, after spray-drying, soluble powders. Chicory flour can be used as an ingredient to improve bread, and, when roasted, chicory is used to enhance the aroma, color, or flavor of food. In many countries, such as Italy, chicory is largely cultivated as a green vegetable, but in Western Europe witloof chicory is mainly consumed due to its slightly bitter taste and whitish-yellow color. One of the quality criteria is the bitter taste caused by sesquiterpene lactones (SLs), which are mainly present in the latex (Sessa et al. 2000). The levels of SLs in chicory is strongly dependent of the cultivar and the cultivation conditions, i.e., climate, season, state of growth or nitrogen, and phosphorus availability (Foster et al. 2006; Peters et al. 1997; Peters and Van Amerongen 1996).

14.1.2.3 *Cynara cardunculus* L.

Cardoon (*Cynara cardunculus*) is an herbaceous plant and a member of the Asteraceae family, which is native to the Mediterranean Basin. This family includes the globe artichoke (*Cynara cardunculus* L. var. *scolymus* L.), the cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC.), and the wild cardoon (*Cynara cardunculus* L. var. *sylvestris* Lamk Fiori) (Rouphael et al. 2016; Scavo et al. 2018). The artichoke crop can last six or more years, with the peak productivity achieved in the third year and more than 60% of the total world production carried out in the Mediterranean area, with Italy and Spain producing 474,000 and 215,000 t per year, respectively (Christaki et al. 2012). In contrast, the cultivation of cardoon is much less important and only has regional significance in the Mediterranean area. The immature inflorescence (or head) of artichoke is consumed due to its high concentration of nutrients and healthy metabolites, which represent 30–40% of the fresh weight, while in the case of cardoon, the young leaves are consumed. The artichoke leaves can also be eaten, and their extracts are sold as dietary products. The main chemicals present in artichoke leaves are phenolics, flavonoids, and sesquiterpenes (Noldin et al. 2003). Aguerin B, grosheimin, cynaropicrin, and 11,13-dihydroxy-8-deoxygrosheimin are the major sesquiterpene lactones isolated from the leaves of cardoon (Rial et al. 2014). Artichoke leaves are frequently used in folk medicine to treat several diseases, including hepatitis, hyperlipidemia, and obesity.

14.1.2.4 *Lactuca sativa* L.

Lettuce (*Lactuca sativa* L.) is a vegetable consumed across the world, and it contains a large amount of beneficial secondary plant metabolites. Spain, Italy, and France produce 35, 21, and 13% tonnage of lettuce production in Europe, respectively. The main types of lettuce produced are criphead, butterhead, looseleaf, and romaine (Barrière et al. 2014). Sesquiterpene lactones impart a bittery taste to lettuce and consumers are able to detect the sweet and bitter compounds in lettuces combined,

with a clear preference for sweeter and less bitter genotypes (and consequently lower sesquiterpene lactone content) (Chadwick et al. 2016). Some of the isolated sesquiterpenes from this species are lactucin, 11β,13-dihydrolactucin, and lactupicrin (Mahmoud et al. 1986). The seeds of lettuce have also been traditionally used in Iran for medicinal purposes, e.g., for the relief of inflammation, gastrodynia, and osteodynia (Sayyah et al. 2004).

14.1.2.5 *Solanum melongena* L.

Aubergine or "eggplant" (*Solanum melongena* L.) is grown in tropical, subtropical, and warm temperate regions for its deep purple or white fruit, which can be up to 30 cm in length and is eaten as a cooked vegetable. Cooked aubergine has a concentration of 2.4 μg/kg of volatiles, and this gives aubergine its characteristic aromatic smell; a monoterpene (car-3-ene) is the main component with an aubergine-like smell (Macleod and De Troconis 1983). One of the major sesquiterpenes reported is aubergenone.

Another vegetable from the Solanaceae family is the tomato. Several sesquiterpenes have been found in their trichomes (Antonious and Kochhar 2003; Falara et al. 2011; Kennedy 2003; Kim et al. 2014; van der Hoeven 2000) although none of these have been reported in the edible mature tomato fruit and they are therefore not covered in this chapter.

14.1.2.6 Cucurbitaceae Family

Pumpkins belong to the Cucurbitaceae family, and they are an edible vegetable found on the American and European continents, as well as in the Caribbean (Gossell-Williams et al. 2006). Three species are representative of this family and are widely cultivated, *Cucurbita maxima*, *Cucurbita pepo*, and *Cucurbita moschata*, with *C. pepo* being the most popular species. In the pumpkin plant, the fruit and the white seeds are the edible part. The pumpkin seed oil contains bioactive compounds such as triterpenoids, sesquiterpenoids, polyphenols, etc. However, sesquiterpenes in pumpkins are rarely mentioned, while other terpenoids are very common (Montesano et al. 2018). The pumpkin seeds yield approximately 50% oil, and they have traditionally been used to treat problems in the prostate gland and irritable bladder (Rajasree et al. 2016).

14.1.2.7 Cyperaceae Family

There are three species of interest regarding nutritional or medicinal value in the Cyperaceae family of monocotyledonous plants: *Cyperus articulatus* L., *Cyperus esculentus* L., and *Cyperus papyrus* L. *C. esculentus* is also known as tigernut or chufa plant, and the tubers are edible and form part of the diet of many people in North Africa and the south of Spain. The essential oils of *Cyperus* plants have diverse interesting biological properties such as analgesic, anti-inflammatory, antipyretic, and antifungal (Hassanein et al. 2014). Some sesquiterpenes have been described for this plant, such as α-cyperone and cyperene, and they will be discussed below.

14.1.3 Algae

For a long time, humans have used algae (or seaweeds) for food and as colorants, fertilizers, medicines, etc. In particular, seaweeds have been consumed as a vegetable since prehistoric times in Eastern Asia, and China is probably the biggest consumer of algae in the world. However, in Western countries, algae are not normally ingested in an unprocessed form. Due to their nutritional value, the interest in algae is still developing. High levels of carbohydrates, minerals, vitamins, and trace elements, such as iodine, have been found in edible algae. Algae have similar or slightly higher levels of fiber than terrestrial foods, and they accumulate a diversity of minerals, which come from their marine surroundings. Furthermore, the lipid content is low and is mainly made up of polyunsaturated fatty acids, numerous antioxidants are present in the shape of vitamins and pigments due to their long exposure to direct sunlight, and the protein content can reach 47% of the dry weight (MacArtain et al. 2008). There are four major algal groups used in food: Chlorophyta (green algae), Phaeophyta (brown algae), Rhodophyta (red algae), and Cyanophyta (blue-green algae) (Bangmei and Abbott 1987). These organisms are a rich source of bioactive secondary metabolites, including sesquiterpenes. These compounds have been described in Chlorophyta, Rhodophyta, and Cyanophyta groups, which will be discussed below. Some of the edible algae species containing sesquiterpenes in each group are listed in the Table 1 (Pallela and Kim 2011; Pereira 2016).

14.1.3.1 Chlorophyta Algae

The characteristic green color of this alga is mainly due to the presence of chlorophylls *a* and *b* in the same ratio as in higher plants. Some *Caulerpa* spp. (Chlorophyta) are edible, and they are consumed in Japan and Indonesia – examples include *C. lentillifera* and *C. racemosa* (aka sea grapes or green caviar). However, other algae might be toxic because of the presence of toxic compounds such as the alkaloid caulerpin (Higa and Kuniyoshi 2000; Nagappan and Vairappan 2014). *Caulerpa peltata* is consumed by natives of the southeast of Iran as a food additive and medicine (Movahhedin et al. 2014). Other green algae, such as *Ulva fasciata* and *U. lactuca*, are food ingredients for soups and salads in India (Chakraborty and Paulraj 2010). Several sesquiterpenes have been isolated from this group, such as caulerpals A and B (from *Caulerpia taxifolia*) (El Gamal 2010) and guai-2-en-10α-ol and guai-2-en-10α-methanol (from *Ulva fasciata*) (Chakraborty et al. 2010).

14.1.3.2 Rhodophyta Algae

Red algae are considered to be the most important source of bioactive secondary metabolites in comparison to the other algal classes. The red color is due to the pigments phycoerythrin and phycocyanin (El Gamal 2010). The genus *Laurencia* (in the Rhodophyta group) comprises red algae including 146 species, which are mainly found in tropical, subtropical, and temperate coastal waters. This fact makes this genus one of the most diverse genera of marine algae. *L. nidifica*, *L. flexilis*, and *L. okamurai* are examples of edible species consumed in countries surrounded by the

Table 1 Edible algae species rich in sesquiterpenes

Algal Group	Species	Common name	Consumer region
Chlorophyta	*Ulva fasciata*	Sea lettuce	Hawaii, Japan, Polynesia
	Ulva lactuca Linnaeus	Sea lettuce	Widespread in Asia, Europe, and Western North America
	Ulva clathrata	Spiky tendrils	USA, China, Japan, Taiwan
	Caulerpa bikinensis W.R. Taylor	–	Polynesia
	Caulerpa lentillifera J. Agardh	Green caviar	Asia, Pakistan, the Philippines, Malaysia
	Caulerpa racemosa var. macrophysa	–	Thailand
	Caulerpa peltata	Big parasol green seaweed	Indonesia, Malaysia, the Philippines, and Tonga
	Caulerpa taxifolia	Lukay-lukay	The Philippines
Rhodophyta	*Laurencia obtusa* (Hudson) J.V. Lamouroux	Rounded brittle fern weed	Indonesia, Thailand, Vietnam, Mediterranean Basin, Hawaii
	Laurencia okamurai	–	Japan
	Laurencia flexilis Setchell	–	China
	Laurencia nidifica J. Agardh	Mustard limu	Hawaii
Cyanophyta	*Arthrospira platensis*	Spirulina	East Africa

Pacific Ocean (Bangmei and Abbott 1987; Harizani et al. 2016; Kim et al. 2008), although several *Laurencia* spp. are commonly consumed as foods or ingredients or for traditional medicine in these countries (Pereira 2016). More than 500 sesquiterpenes have been described for this genus (Harizani et al. 2016).

14.1.3.3 Cyanophyta Algae

Cyanophyta are one of the most primitive forms of photosynthetic prokaryotes. They are also called "blue-green algae," although the term "algae" in modern usage is restricted to eukaryotes. Cyanophyta have been used as food or medicine for centuries, and the first recorded human consumption is from the fourteenth century. It has been reported that the consumption of cyanophyta promotes immunity and prevents inflammatory diseases (Siah Ku et al. 2013). Within this group of algae, spirulina or *Arthrospira platensis* is used for its nutritional value and reported medicinal properties, such as antidiabetic, antihypertensive, and antimicrobial actions. The sesquiterpenes β-caryophyllene and β-selinene have been reported for this species.

14.1.4 Food with Medicinal Properties

Medicinal plants have been used since ancient times to treat several disorders and diseases. Some of these natural medicines have become part of our diet as functional

foods that provide medicinal effects in addition to the nutritive and taste values. Different edible medicinal plants found to date will be discussed in this section, and these contain sesquiterpenes as major compounds.

14.1.4.1 Common and Japanese Butterbur

Common butterbur (*Petasites hybridus* (L.) G. Gaertn., B. Mey. & Scherb) is an herbaceous perennial plant from the Asteraceae family. The extracts from rhizomes and leaves have been used as dietary supplements as they have shown an ability to reduce the occurrence or severity of migraines and provide an effective treatment for hay fever. In traditional medicine such extracts have been used as spasmolytic and anti-inflammatory agents (Avula et al. 2012). Likewise, Japanese butterbur (*Petasites japonicus* Maxim., Compositae family) is a perennial herbaceous plant that is cultivated as a vegetable in Japan. Several sesquiterpene lactones have been isolated from this plant, for instance, fukinone and 2β-hydroxyfukinone. Furthermore, fresh stems from Japanese butterbur are used as a food garnish in Japanese dishes (Yoshikawa et al. 2006). In traditional medicine, well-backed flower bud has been used as an expectorant or in the treatment of asthma. Moreover, juice squeezed from the leaves has been used to treat bee stings (Shimoda et al. 2006).

14.1.4.2 *Ginkgo biloba* L.

Ginkgo biloba (Ginkgoaceae family) has been used in Chinese medicine at least since the fourteenth century for the treatment of asthma, bronchitis, and cardiovascular diseases. The German physician-pharmacist Dr. Willmar Schwabe III introduced this plant into medical practice in the west, with the first standardized *G. biloba* leaf extract used in 1965 for the management of insufficiency states of cerebral and peripheral circulation and neurosensory organs. Currently, this is one of the most widely sold herbal supplements and medicines in the world, and it has become one of the top-selling dietary supplements in the USA (Avula et al. 2015). In China, extracts of *G. biloba* leaves are formulated as tablets and capsules for oral administration and as injections for intravenous administration. The popularity of this supplement stems from it having positive effects as add-on therapies, on memory and on the circulatory system in clinical studies; it improves cognitive function and may be beneficial in patients with Alzheimer's disease (Ahlemeyer and Krieglstein 2003; Janßen et al. 2010). The active components include ginkgolides A, B, C, and J (diterpene lactones), bilobalide (sesquiterpene lactone), and flavonol glycosides. *Ginkgo* extracts are available as tinctures, capsules, tablets, decoction, and a standardized extract, which contains 24–32% flavonoids and 6–12% terpenoids (Zengion and Yarnell 2011).

14.1.4.3 *Chrysanthemum parthenium* (L.) Bernh.

Feverfew plant (*Chrysanthemum parthenium* (L.) Bernh.) is also frequently referred to as *Tanacetum parthenium* (L.) Sch. Bip. This plant is common in gardens and has long been used as a folk remedy in northern Europe for the treatment of various diseases, including arthritis, fever, and migraine. It is consumed by eating fresh feverfew leaves grown domestically or as tablets made from dried powdered feverfew leaves, which is widely available in health food shops in the United Kingdom

(Summer et al. 1992). The main component responsible for the biological activity of feverfew preparations is the sesquiterpene lactone parthenolide. In addition, this plant also contains phenolic compounds that show anti-inflammatory activity. These properties and the good source of bioactive compounds provide an opportunity to develop beverages based on this plant. The optimal conditions for the manufacture of feverfew infusions were studied by Marete et al. (Marete et al. 2011). Parameters such as color stability, stability of phenolic compounds and parthenolide, as well as storage time were studied. This study showed that the most favorable conditions to prepare feverfew infusions are mildly acidic (pH = 4.6) and refrigerated storage maintains stable phenolic and parthenolide contents for approximately 4 months. Moreover, feverfew extracts have also shown inhibition in the generation of prostaglandins from exogenous arachidonic acid in a cell-free system, the secretion of histamine from activated mast cells, and the aggregation and secretion of 5-hydroxytryptamine from platelets (Summer et al. 1992).

14.1.4.4 *Artemisia annua* L., *Artemisia absinthium* L., and *Artemisia herba-alba* Asso.

The genus *Artemisia* (family of Asteraceae) includes more than 500 species. There are several reports in which *Artemisia* plants are described as dietary foods and as traditional herbal medicines for the treatment of diseases such as malaria, hepatitis, cancer, and inflammation (Bourgou et al. 2017). For instance, *Artemisia annua*, known as "qinghao" in traditional Chinese medicine, is used in the treatment of malaria, and *Artemisia herba-alba* – known as "chih" in Tunisia – is an aromatic and medicinal herb commonly used to aromatize tea and in local folk medicine for the treatment of diabetes, coughing, intestinal disturbances, parasitic infection, and neurological diseases. On the other hand, *Artemisia absinthium* L. (also known as wormwood) is an edible vegetable and traditional medicinal food in East Asia. It is a yellow flowering, perennial plant, and it is used for the treatment of several illnesses including chronic bronchitis, asthma, gastroenteritis, and pruritus, and it also has sedative effects (Aberham et al. 2010). The aerial parts are commonly used in gastric herbal preparations, dietary supplements, and alcoholic beverages (as absinthe products) (Abad et al. 2012). Furthermore, some sesquiterpenes from this plant have been reported to provide the characteristic bitterness, for instance, ketopelenolide b (Aberham et al. 2010).

14.1.4.5 *Ambrosia artemisiifolia* L.

Common ragweed (*Ambrosia artemisiifolia* L., Asteraceae) is an annual invasive plant with high allergenic potential that triggers rhinitis, ocular rhinitis, and other symptoms of hay fever (33 million people are sensitized in Europe and 23 million in the USA). However, this plant was used by Native Americans for medicinal purposes. Recently, the herb of *A. artemisiifolia* has gained popularity as a medicinal plant and food. Several products are available on the market, typically as food supplements (dry ragweed powder, alcoholic extract) or as food (puree made of the fresh buds of the plant). Twenty-nine sesquiterpenes have been isolated from *A. artemisiifolia*, and many of them have shown antibacterial, antifungal, antiprotozoal,

anti-inflammatory, cardiovascular, and hepatoprotective effects. The beneficial effects of ingested ragweed include anxiolytic activity, strengthening of the immune system, detoxification of the body, improved erectile function, appetite stimulation, and anticarcinogenic, anti-allergic, and mucolytic effects. However, recent studies have shown that the repeated use of ragweed resulted in toxic effects in rats, and these results question the safety of long-term human consumption of common ragweed (Kiss et al. 2017).

14.1.4.6 *Taraxacum officinale* (L.) Weber ex F. H. Wigg.

The common dandelion (*Taraxacum officinale)* is a plant of the Asteraceae family, and it is used as a food and medical herb due to various health benefits, which include antioxidant, anti-inflammatory, and anticarcinogenic properties. Moreover, dandelion is a completely edible weed that is consumed raw in salads, cooked, roasted (used as a coffee substitute), or as a flavoring, for example, in alcoholic drinks, candies, and cheeses. Different dandelion tissues contain sesquiterpene lactones that are responsible for the bitter taste (Aberham et al. 2010; Esatbeyoglu et al. 2017).

14.1.4.7 *Chrysanthemum indicum* L.

Chrysanthemum indicum L. (Compositae family) is a perennial garden herb used to treat several immune-related disorders, hypertension symptoms, and infectious diseases in Korean and Chinese medicine (Hwang and Kim 2013). The flowers are edible and are also commonly used as tea to cure inflammation, headache, and eye diseases (Morikawa 2007). Several sesquiterpenes have been isolated from the methanolic extract of these flowers, for instance, indicumolides A, B, and C (Feng et al. 2009).

14.1.4.8 *Litsea cubeba* (Lour.) Pers.

Litsea cubeba (Lour.) Pers. (Lauraceae family) can be used as a flavoring or herbal medicine. It is distributed in Southeastern Asia, Southern China, Japan, and Taiwan. Its fruit is known as "Chen-Qie-Zi" in Chinese traditional medicine, and it has been used due to its wide range of biological activities, which include antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory properties (Si et al. 2012; Wang and Liu 2010). This fruit possess a characteristic flavor that resembles a mixture of pepper, ginger, and citrus. For this reason, the essential oil of its fruit is used as a flavor enhancer in foods, cosmetics, and cigarettes and as a raw material to produce citral. Sesquiterpene hydrocarbons (β-caryophyllene, α-copaene, β-elemene, α-humulene, and δ-cadinene) and oxygenated sesquiterpene (caryophyllene oxide) have been identified in the essential oil (Chen et al. 2016; Yang et al. 2013). Nevertheless, the main compounds that constitute the essential oil are monoterpenes (94–98%). Likewise, this composition depends on geographical and climatic conditions. Moreover, the biological activities described for this essential oil are attributed to synergistic or antagonistic effects of its chemical composition (Si et al. 2012).

14.1.4.9 *Thymus saturejoides* Coss.

Thymus saturejoides Coss. (commonly named in Morocco as "za-itra") is one of the most popular aromatic herbs distributed in the Mediterranean basin. It is a flavoring agent used in herbal tea, and it is appreciated for its tonic and stimulant properties as well as being used for the preservation of a range of food products. Numerous biological properties have been reported for this plant, such as analgesic, anti-inflammatory, antispasmodic, and antioxidant effects (Kasrati et al. 2014).

14.1.4.10 *Warburgia ugandensis* Sprague and *W. stuhlmannii* Engl.

Sesquiterpenes have been described as being responsible for the bitter and hot taste of many plants. For instance, *Warburgia ugandensis* Sprague and *W. stuhlmannii* Engl., which are often used as food spices and medicines, are very hot to human taste and polygodial, warburganal, muzigadial, and ugandensidial (Fig. 2) have been described as being responsible for this taste. These compounds are produced by the plant as antifeedant compounds against larvae of armyworms (Kubo and Ganjian 1981).

14.1.5 Mushrooms

Since ancient times mushrooms have been consumed by humans as part of the normal diet due to the high nutritional value and their attractive taste and aroma. Some researchers have indicated that edible mushrooms are favorably nutritional when compared with meat, egg, and milk as food sources, with a protein content in the range 27–48%, fewer than 60% carbohydrates and low fat content (2–8%) (Wani et al. 2010). Besides, medicinal benefits have been reported for mushrooms in addition to the nutritional value, including antitumor, antiviral, and hypolipidemic effects (Mattila et al. 2000). Despite the beneficial properties of mushrooms, sesquiterpene lactones have only been described in the *Antrodia camphorata* species.

14.1.5.1 *Antrodia camphorata*

Antrodia camphorata – also known as "niuchangchih" or *Antrodia cinnamomea* – is an edible Taiwanese mushroom that grows in the *Cinnamomum kanehirae* tree trunk. This mushroom is widely used as a food dietary supplement for cancer prevention. In the past, *A. camphorata* has been used in traditional medicine to

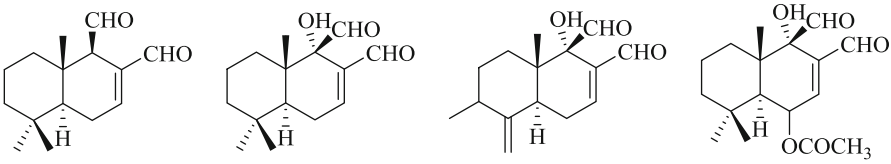


Fig. 2 Sesquiterpenes responsible for the bitter taste of *Warburgia ugandensis* and *W. stuhlmannii* species

treat influenza, headache, and fever, but numerous recent studies have led to the discovery of different applications for this mushroom. Nowadays, extracts of *A. camphorata* are mainly used as functional food due to the many pharmaceutical effects. More than one hundred secondary metabolites have been isolated from this species, and these include the sesquiterpenoid antrocin, which has been shown to be the most active. *A. camphorata* extract has shown many pharmacological activities such as anticancer activity, anti-inflammatory and hepatoprotective properties, and antiviral and immunomodulatory effects, among others (Geethangili and Institute 2006).

14.2 Bioactive Constituents

Sesquiterpenes are colorless lipophilic components, and their biosynthesis involves isoprene units and occurs through farnesyl pyrophosphate found in endoplasmic reticulum. It is well established that in higher plants, two independent pathways, from different compartments, are involved in the biosynthesis of IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate). In the cytosol, IPP is derived from the mevalonic acid (MVA) pathway, which begins with the condensation of acetyl-CoA. It is well established that in this cytosolic compartment, the production of sesquiterpenes and triterpenes takes place. Moreover, in plastids IPP is generated in the methylerythritol phosphate (MEP) pathway, and it is assumed that in this compartment, monoterpenes, diterpenes, and triterpenes are produced. Nevertheless, there is evidence that cross talk occurs between the two different biosynthetic pathways (Fig. 3; Yu and Utsumi 2009).

14.2.1 Bioactive Sesquiterpenes with Low Levels of Functionalization (Essential Oils)

Sesquiterpenes with few oxygenated functions in the structure, i.e., farnesenes or bisabolenes (Fig. 4), are normally present in the essential oils and aromas of plants. Many of them are phytoalexins, compounds produced by plants in response to biotic stress or antifeedants (Dai et al. 2015). Sesquiterpenes present in essential oils from different foods are listed in Table 2.

The sesquiterpene α-farnesene is a pale yellow to yellow liquid with a vegetative scent, a taste of green vegetables, and a fruity aftertaste. It is used as a flavoring agent in food and beverages, and it imparts the characteristic odor to green apple. Several studies have demonstrated that essential oils from green apples play an important role in the reproduction and olfactory behaviors of moth females. α-Farnesene – among other components – has been shown to play an important role in the periodicity of reproductive behaviors and olfactory responses (Yan et al. 2003).

Moreover, α-copaene, δ-cadinene, and valencene constitute the major sesquiterpenes found in oranges. The sesquiterpene α-copaene appears in a large variety of plants and spices, such as sweet basil and black pepper, as well as in several citrus

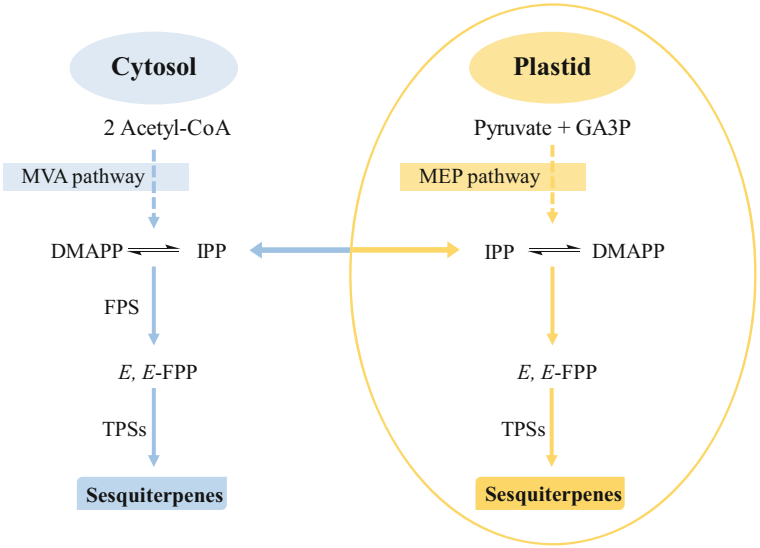


Fig. 3 Intercrossed biosynthetic pathways for sesquiterpenes. (Image adapted from Yu and Utsumi 2009)

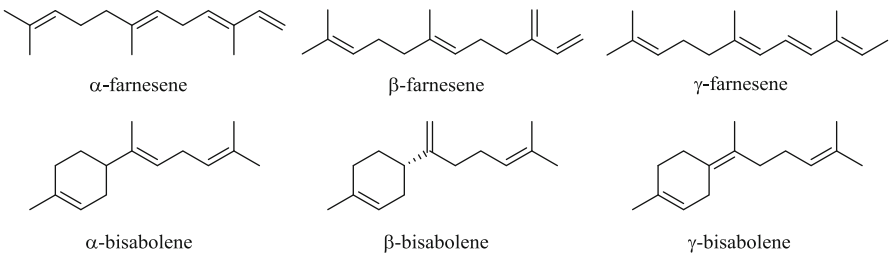


Fig. 4 Several examples of farnesene and bisabolene sesquiterpenes

fruits. The aroma of α-copaene is woody with notes to spicy honey. Essential oils that contain α-copaene have shown diverse biological activities, including antibacterial, antimicrobial, fungicidal, anti-inflammatory (Sádecká et al. 2014), and antitumor (Yang et al. 2017). δ-Cadinene is a bicyclic sesquiterpene that is insoluble in water and is a remarkable intermediate in the biosynthesis of important phytoalexins. This compound brings a sharp aroma to dry wood. It has been reported that *Rhododendron nivale* Hook. essential oils (in which the main component is δ-cadinene) showed in vitro acaricidal activity against adult *Psoroptes cuniculi* in a concentration- and time-dependent manner. In the same way, essential oils from

Table 2 Sesquiterpenes present in the essential oils of food

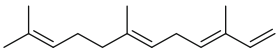
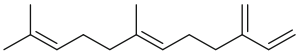
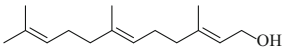
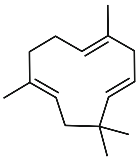
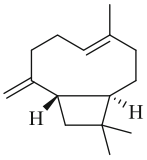
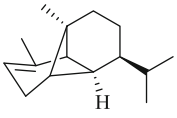
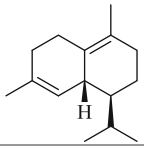
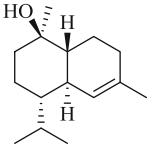
Name	Food	Reference	Structure
(E,E)-α-Farnesene	Apple; peach pear	Liu et al. (2017), Pechous and Whitaker (2004)	
β-Farnesene	Grape, *L. cubeba*, *C. japonica*	Okuno et al. (2017); Perestrelo et al. (2014), Si et al. (2012)	
Farnesol	Grape, *P. eryngii*	Perestrelo et al. (2014), Usami et al. (2014)	
α-caryophyllene / α-humulene	*Grape*, *L. cubeba*, *C. articulatus*, *C. esculentus*, *C. papyrus*	Chen et al. (2016), Hassanein et al. (2014), Kubmarawa et al. (2005), Perestrelo et al. (2014), Si et al. (2012)	
β-Caryophyllene	Orange, grape, *A. annua*, *C. indicum*, *L. cubeba*, *L. obtusa*, *C. articulatus*, *C. esculentus*, *C. papyrus*, *F. velutipes*	Chen et al. (2016), Demirel et al. (2011); Duke et al. (1987), Hassanein et al. (2014), Högnadóttir and Rouseff (2003), Hwang and Kim (2013), Li et al. (2018), Perestrelo et al. (2014)	
α-Copaene	Orange, pineapple, *L. cubeba*, *C. articulatus*, *C. esculentus*, *C papyrus*, *P eryngii*	Chen et al. (2016), Hassanein et al. (2014), Hunter and Brogden (1965), Lamikanra and Richard (2004), Usami et al. (2014)	
δ-Cadinene	Orange, *L. cubeba*, *C. articulatus*, *C. esculentus*, *P. eryngii*	Chen et al. (2016), Hassanein et al. (2014), Hunter and Brogden (1965), Usami et al. (2014)	
α-Cadinol	*C. indicum, L. cubeba*, *T. polium*, *C. articulatus*, *C. esculentus*	Hassanein et al. (2014), Hwang and Kim (2013), Nakatsu et al. (2000), Si et al. (2012)	

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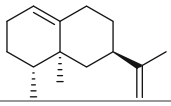
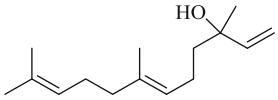
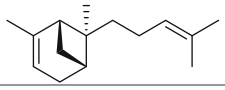
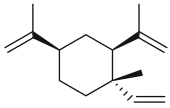
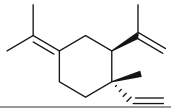
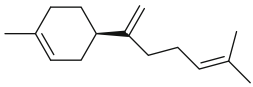
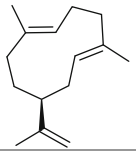
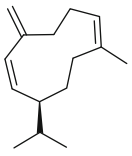
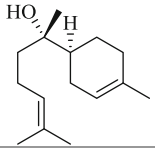
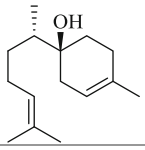
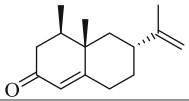
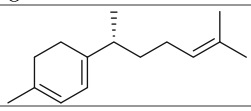
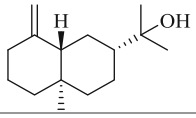
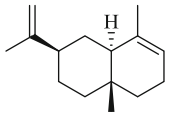
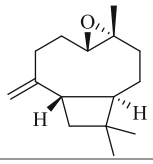
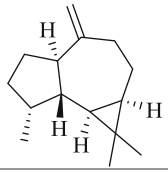
Table 2 (continued)

Name	Food	Reference	Structure
Valencene	Oranges, *L. obtusa*	Demirel et al. (2011), Hunter and Brogden (1965)	
Nerolidol	Strawberry; grape, *L. cubeba*, *L. obtusa*	Aharoni et al. (2005), Demirel et al. (2011), Liu et al. (2017), Perestrelo et al. (2014), Wang and Liu (2010)	
α-Bergamotene	Lemon	MacLeod et al. (1966)	
β-Elemene	Grape, *L. cubeba*, *C. japonica*, *L. obtusa*, *C. articulatus*, *C. esculentus*, *F. velutipes*	Chen et al. (2016), Demirel et al. (2011), Hassanein et al. (2014), Li et al. (2018), Okuno et al. (2017), Perestrelo et al. (2014)	
γ-Elemene	*L. cubeba*, *P. eryngii*	Si et al. (2012), Usami et al. (2014)	
β-Bisabolene	Grape, *A. annua*, *L. cubeba*, *P. eryngii*	Duke et al. (1987), Perestrelo et al. (2014), Si et al. (2012), Usami et al. (2014)	
Germacrene A	*L. cubeba*	Si et al. (2012)	
Germacrene D	Grape, *C. indicum*, *L. cubeba*, *C. japonica*, *C. articulatus*, *C. esculentus*, *C. papyrus*	Hassanein et al. (2014), Hwang and Kim (2013), Okuno et al. (2017), Perestrelo et al. (2014), Si et al. (2012)	
α-Bisabolol	Grape	Perestrelo et al. (2014)	

(continued)

Table 2 (continued)

Name	Food	Reference	Structure
β-Bisabolol	*A. ordosica*, *C. articulatus*, *C. esculentus*	Abad et al. (2012), Hassanein et al. (2014)	
Nootkatone	Grapefruit	Kfoury et al. (2017)	
γ-Curcumene	*C. indicum*	Hwang and Kim (2013)	
β-Eudesmol	*T. polium*, *D. japonica*, *L. obtusa*	Demirel et al. (2011), Nakatsu et al. (2000)	
α-Selinene	*C. japonica*, *L. obtusa*, *C. esculentus*	Demirel et al. (2011), Hassanein et al. (2014), Okuno et al. (2017)	
Caryophyllene oxide	*L. cubeba*, *C. articulatus*, *C. esculentus*, *F. velutipes*	Si et al. (2012)	
Aromadendrene	*L. obtusa*, *C. articulatus*, *C. esculentus*	Demirel et al. (2011), Hassanein et al. (2014)	

Xenophyllum poposum induce in vitro antibacterial and antifungal activities (Tracanna et al. 2012). Additionally, in vitro antiproliferative and apoptotic effects on human ovarian tumoral cell line (OVCAR-3) have been described for δ-cadinene (Hui et al. 2015). Finally, valencene is a hydrophobic sesquiterpene with an eremophilane skeleton, and it is found in diverse citrus species, such as mandarin or oranges, and in other aromatic plants like rosemary or common oregano. The odor description suggests sweet and fresh citrus, reminiscent of grapefruit, woody and orange aroma. Significant cytotoxic effects on HeLa cell line and antioxidant activity have been reported for this sesquiterpenoid (Liu et al. 2012).

Nerolidol (in addition to linalool) is the main terpenoid in cultivated strawberry (Aharoni et al. 2005), and it can also be found in grape berry and in wine

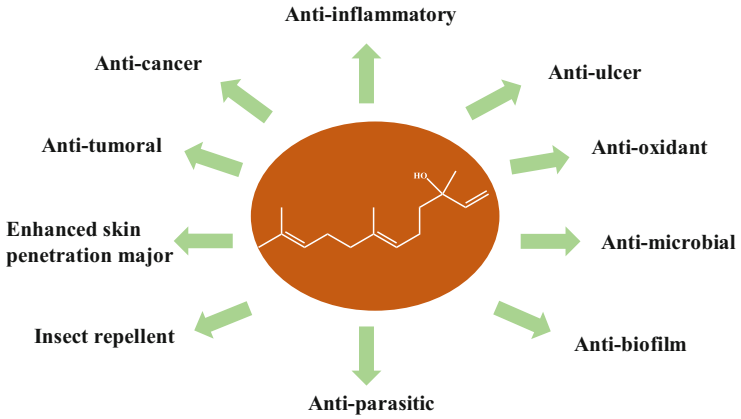


Fig. 5 Biological activities of nerolidol

(Perestrelo et al. 2014). Nerolidol is one of the major compounds present in the essential oils of several plants, e.g., *Cananga odorata* (ylang-ylang) and hemp. In fact, researchers are focused on the biological activities of essential oils that have been isolated from wood, leaves, and other aerial parts of plants and from bulbs and seeds from diverse genera of different families such as *Piperaceae*, *Amaranthaceae*, *Capparaceae*, and *Amaryllidaceae*. Several biological activities have been described, and these are illustrated in Fig. 5. It has been reported that *cis*-nerolidol exhibits weaker activity than its *trans* conformer (Weng-Keong et al. 2016).

Farnesol is a hydrophobic sesquiterpene alcohol that can be ingested from diverse fruits, including grapes (Perestrelo et al. 2014), tomatoes, or peaches (Špicáková et al. 2017) and infusions of chamomile or lavender (Duncan and Archer 2008). Its delicate aroma is reminiscent of lilac, and it is therefore widely used in perfumes. Diverse bioactivities have been described for farnesol, including anti-inflammatory, anti-allergic, antioxidant, chemopreventive, and anticancer properties.

Nootkatone, or (+)-nootkatone, is the most important and expensive sesquiterpene in grapefruit because it provides the characteristic aroma and flavor responsible for the citrus, sweet, and woody nuance. With an eremophilane backbone, nootkatone is also found in Alaska yellow cedar trees or *Cupressus nootkatensis* and in the roots of *Chrysopogon zizanioides* (a bunchgrass native to India), the essential oil of which is known as tranquility oil due to its sedative effects. Moreover, it has been described in Alpiniae Oxyphyllae Fructus, a Chinese plant used in folk medicine to improve memory. Other traditional uses highlight the insecticidal activity of nootkatone. High industrial demand for nootkatone has led to the development of synthetic and biosynthetic routes. In this respect, the biochemical oxidation of valencenes has been described, and the production of nootkatone by modified microorganisms has been reported (Sowden et al. 2005). Additionally, nootkatone has shown considerable antiproliferative effects against two human lung

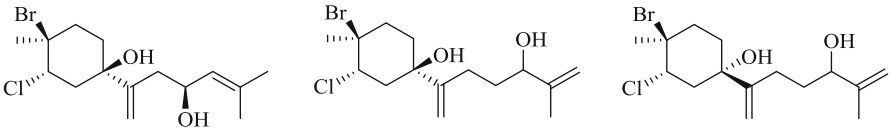


Fig. 6 β-Bisabolenes isolated from *L. scoparia*

adenocarcinoma and human promyelocytic leukemia cell lines (Gliszczynska et al. 2011) and anti-inflammatory activity due to its involvement in the expression of diverse cytokines such as TNF-α, which is associated with Alzheimer's disease (Y. Wang et al. 2018).

Three β-bisabolene-type compounds were isolated from *L. scoparia* (Davyt et al. 2006): (2*S*,3*S*,6*R*,9*S*)-3-Bromo-2-chloro-2,3-dihydro-6,9-dihydroxy-β-bisabolene [(1*R*,3-*S*,4*S*)-4-bromo-3-chloro-1-((*S*)-4-hydroxy-6-methylhepta-1,5-dien-2-yl)-4-methylcyclohexanol], (2*S*∗,3*S*∗,6*R*∗)-3-bromo-2-chloro-2,3-dihydro-6,10-dihydroxy-β-bisabolene[(1*R*∗,3*S*∗,4*S*∗)-4-bromo-3-chloro-1-(5-hydroxy-6-methylhepta-1,6-dien-2-yl)-4-methylcyclohexanol], and (2*S*∗,3*S*∗,6*S*∗)-3-bromo-2-chloro-2,3-dihydro-6,10-dihydroxy-β-bisabolene[(1*S*∗,3*S*∗,4*S*∗)-4-bromo-3-chloro-1-(5-hydroxy-6-methylhepta-1,6-dien-2-yl)-4-methylcyclohexanol] (Fig. 6). The first compound mentioned showed moderate antiparasitic activity against *Nippostrongilus brasiliensis* with an EC_{50} value of 0.11 mM.

14.2.2 Bioactive Sesquiterpenes Containing the α-Methylene-γ-lactone Moiety

The bioactive constituents of several sesquiterpenes contain an α-methylene-γ-lactone moiety in their structure, and this has been designated as being responsible for the activity by reacting through a Michael addition with free sulfhydryl or amino groups in proteins to alkylate them (Ghantous et al. 2010). Such compounds have been described as cytotoxic, antitumor, allergic, antimicrobial, antifeedant, phytotoxic, and insecticidal agents. Sesquiterpene lactones can be classified into several main classes, including germacranolides, eudesmanolides, guaianolides, pseudoguaianolides, heliangolides, and hypocretenolides (Fig. 7; Chaturvedi et al. 2015). For instance, parthenolide is isolated from feverfew (*Tanacetum parthenium*), which has been proposed as a source of bioactives for functional foods (Marete et al. 2011), and it showed anti-inflammatory, antimicrobial, and antitumor activity (Nakshatri et al. 2004; Won et al. 2004). Isoalantolactone, isolated from ragweed, has been reported to possess multiple biological and pharmacological activities including antibacterial, antifungal, anti-inflammatory, and anticancer effects (Fig. 8; Kiss et al. 2017).

Sesquiterpene lactones are also responsible for the bitter taste of many plants, for instance, dandelion tissues contain sesquiterpene lactones such as eudesmanolides, namely, tetrahydroridentin B and taraxacolide-*O*-β-glucopyranoside; guaianolides,

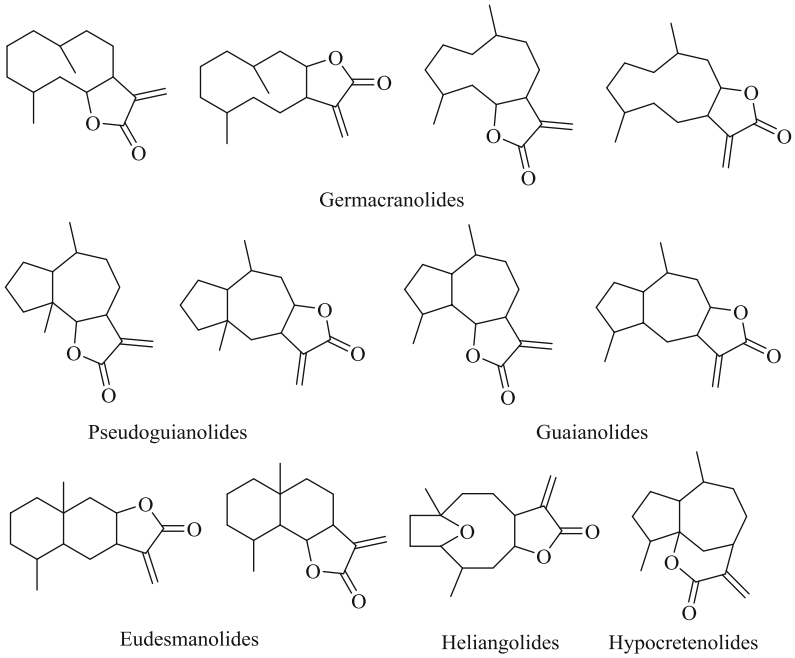


Fig. 7 Classification of sesquiterpene lactones depending on the type of backbone

namely, 11β,13-dihydrolactucin and ixerin D; and esterified germacranolide acids, namely, taraxinic acid β-glucopyranosylester. These compounds have shown a wide range of biological activities, including anti-inflammatory, antimicrobial, hypolipidemic, and antihyperglicemic properties. On the other hand, chicory also contains this particular bitterness due to the presence of lactucin, 8-deoxylactucin, lactucopicrin, and their 11(*S*),13-dihydro derivatives (Fig. 9; Willeman et al. 2014).

Cynaropicrin is the major compound found in *Cynara* species. Besides its anti-inflammatory properties (Mizuno and Usuki 2018), it has also shown phytotoxic activity on two weed species of economic importance, i.e., barnyard grass (*Echinochloa crus-galli* L.) and *Brachiaria* (*Urochloa decumbens* (Stapf) R.D. Webster) (Rial et al. 2014). Root length was the most affected parameter with values of 80% of inhibition achieved at the first two concentrations tested (1000 and 300 μM). Furthermore, the joint action of several sesquiterpenes found in extracts of *Cynara* species has been evaluated using the etiolated wheat coleoptile bioassay. The sesquiterpenes studied were aguerin B, grosheimin, cynaropicrin, and 11,13-dihydroxy-8-desoxygrosheimin (Rial et al. 2016). Synergism, additivity, and antagonism were found depending on the compound and the relative proportions in the mixture. For example, the compound 11,13-dihydroxy-8-deoxygrosheimin showed synergism and additivity with aguerin B at high and low doses, respectively, whereas with grosheimin, it showed additivity at all tested doses, and with cynaropicrin it showed antagonist effects.

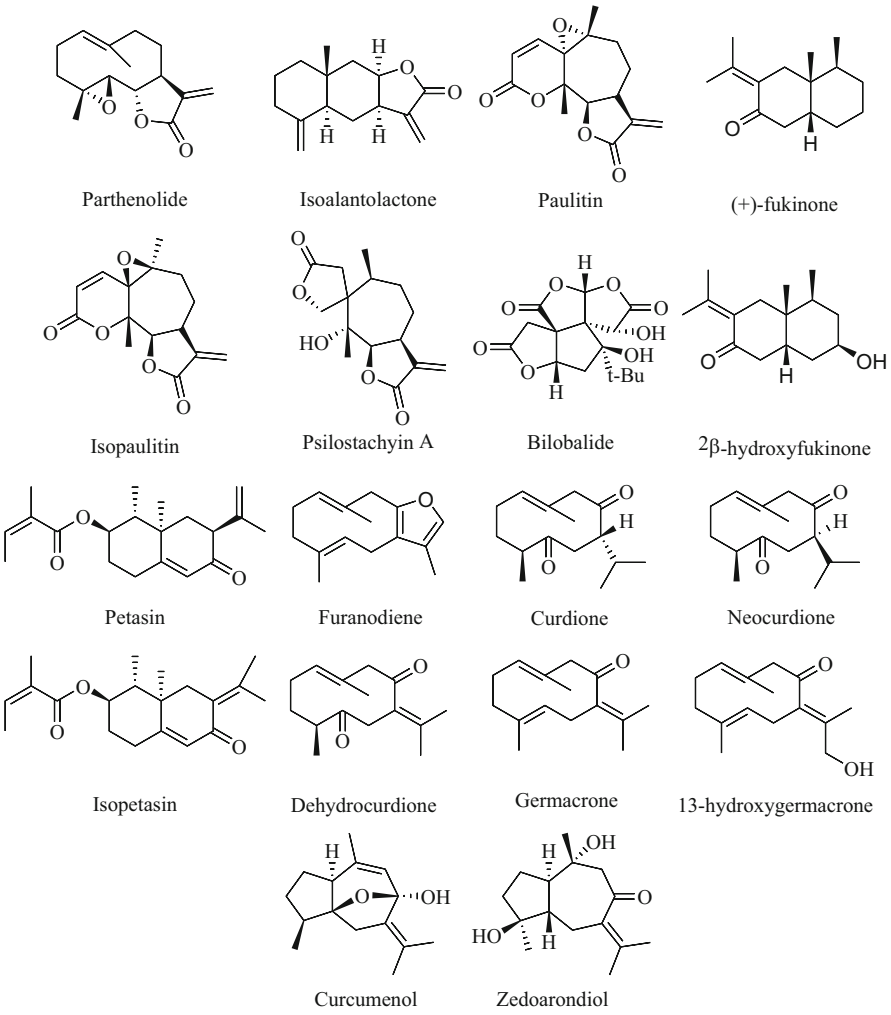


Fig. 8 Bioactive sesquiterpenes from natural sources

14.2.3 Bioactive Sesquiterpenes Containing More than One Lactone Ring

Sesquiterpenes with more than one lactone ring have been identified as bioactive compounds. For instance, paulitin and isopaulitin have shown anticancer activity, and psilostachyin A has shown antileishmanial and antiplasmodial activities (Fig. 8; Kiss et al. 2017).

One of the compounds responsible for the activity of *Ginkgo biloba* is bilobalide. Bilobalide is a sesquiterpene trilactone (Fig. 8) that has shown beneficial effects on cerebral ischemia and neurodegenerative disorders. Several mechanisms of action

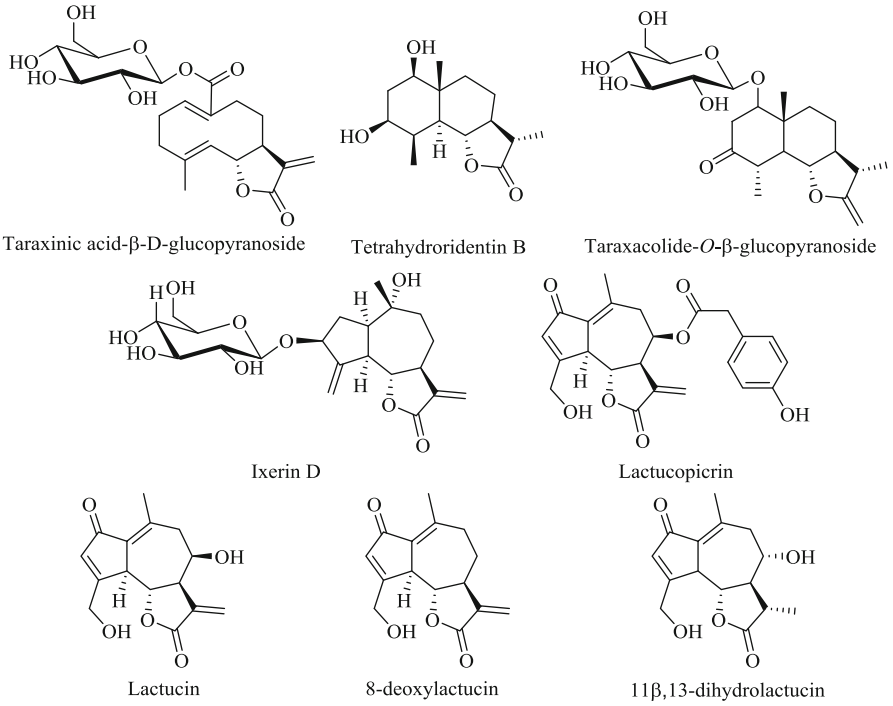


Fig. 9 Sesquiterpenes responsible for the bitter taste in dandelion and chicory

have been described for its neuroprotective properties, including preservation of mitochondrial ATP synthesis and suppression of hypoxia-induced membrane deterioration in the brain, among others (Defeudis 2002).

14.2.4 Bioactive Glycosylated Sesquiterpenes

Glycosylated sesquiterpenes are found, for instance, taraxinic acid or ixerin D has been identified in dandelion (Fig. 10), and such compounds are normally isolated with a glucose molecule, which improves their solubility in water. Moreover, glycosylated sesquiterpene lactones have been identified in chicory and related species, e.g., picriside A, cichorioside B, and crepidiasides A and B (Fig. 10; Peters et al. 1996). These compounds have shown anti-inflammatory properties (Wirngo et al. 2016).

14.2.5 Bioactive Sesquiterpenes Without a Lactone Ring

Sesquiterpenes that do not contain a lactone ring have also proven to be active. For instance, petasin and isopetasin (Fig. 8) are responsible for the activity of butterbur

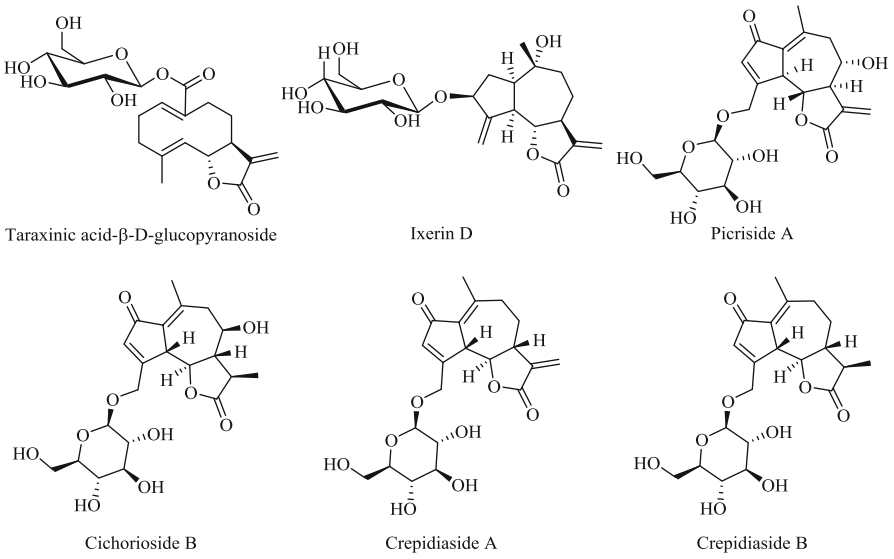


Fig. 10 Glycosylated sesquiterpenes

extract. These compounds are effective in the treatment of migraine headache and hay fever (Schapowal 2002; Sutherland and Sweet 2010). These compounds have also been used as spasmolytic and anti-inflammatory agents. In addition, (+)-fukinone and 2β-hydroxyfukinone (Fig. 8) appear to be involved in the anti-type I allergic mechanism of butterbur extract and have shown inhibitory effects on mast cell degranulation (Shimoda et al. 2006).

Moreover, the sesquiterpenes furanodiene, curdione, neocurdione, dehydrocurdione, germacrone, 13-hydroxygermacrone, curcumenol, and zedoarondiol (Fig. 8) have been described as being responsible for the hepatoprotective effect of *Curcuma zedoaria* extracts (Morikawa et al. 2002).

It is worth highlighting a mixture of five sesquiterpenes produced when aubergine is incubated with spores of *Monilinia fructicola* or other fungi. These compounds are not found when this vegetable is incubated in water (Stoessl et al. 1975). These sesquiterpenes are stress metabolites that may function as a natural defense (Fig. 11).

Additionally, cyperone, cyperene, and patchoulenone from *Cyperus* species are considered to be the main biologically active compounds in its essential oils (Fig. 12).

Bioactive constituents classified within this group have also been found in algae. For instance, caulerpenyne is the major secondary metabolite produced by Chlorophyta *C. taxifolia*, and it is present in other edible *Caulerpa* spp. (Fig. 13). This compound has anticancer, antitumor, and antiproliferative properties (Smit 2004). In particular, this compound is cytotoxic on the neuroblastoma SK-N-SH cell line with a maximum effect after 24 h of treatment with $IC_{50} = 8 \pm 1$ μM. In the

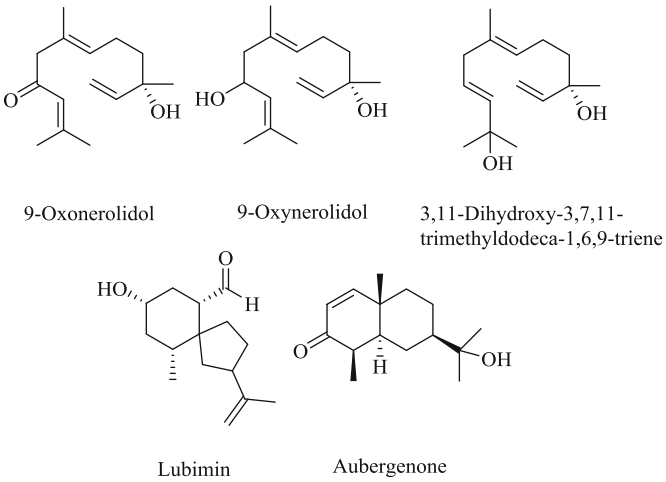
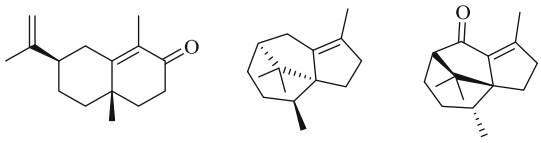


Fig. 11 Stress metabolites produced by aubergine

Fig. 12 Biologically active sesquiterpenes from *Cyperus* species



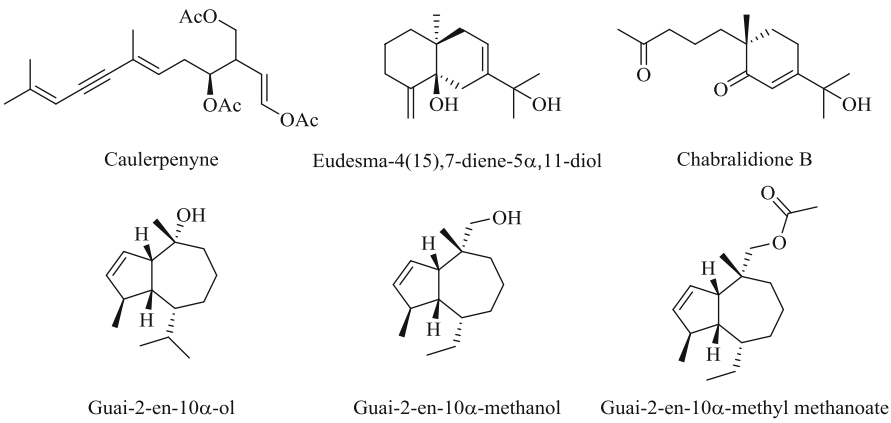


Fig. 13 Bioactive sesquiterpenes found in algae

neuroblastoma cell line at 10 μM and in 2 h, caulerpenyne induced cell death but not mitotic arrest in G_2/M phase. The microtubule network of SK-N-SH cells was also affected by this metabolite, which condensed the microtubules at the cell periphery and made the neurites disappear.

Eudesman-4(15),7-diene-5,11-diol and *seco*-eudesmane chabrolidione B (both from *L. obtusa*) (Fig. 13) have shown significant antifungal activity, with an MIC value of 2.92 μM for both compounds against *Candida albicans* and 2.10 and 4.10 μM for eudesma-4(15),7-diene-5α,11-diol and the *seco*-eudesmane chabrolidione B, respectively, against *C. tropicalis* (Alarif et al. 2016).

In addition, three guaianolides isolated from *U. fasciata* were active against three different *Vibrio* spp. (*V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus*). Among them, guai-2-en-10α-methyl methanoate had the highest activity against *V. parahaemolyticus* and *V. vulnificus* (MIC 25 μg/mL for each), while guai-2-en-10α-ol, with the cycloheptanol moiety, exhibited the lowest activity of the three against *V. parahaemolyticus* and *V. harveyi* (MICs 110 and 160 μg/mL, respectively) (Chakraborty et al. 2010).

14.2.6 Bioactive Sesquiterpenes with a 1,2,4-Trioxane Ring

The Chinese medicinal plant *Artemisia annua* L. (qinghao) is the source of the sesquiterpene lactone artemisinin (also called qinghaosu) and of a large number of natural products, including amorphane and cadinane sesquiterpenes. Artemisinin is an effective antimalarial agent, especially for multidrug resistance and cerebral malaria. The unusual 1,2,4-trioxane ring structure that makes this natural product unique (Fig. 14) is responsible for this activity, and it is the most potent antimalarial available, rapidly killing all asexual stages of *Plasmodium falciparum* (Eckstein-Ludwing et al. 2003). Due to its activity against the malarial parasite, artemisinin has attracted the attention of the World Health Organization (WHO) for use as an antimalarial drug. However, the therapeutic value of this compound is limited by its low solubility in both oil and water and by the low content in *A. annua*, which ranges from 0.01% to 0.8% of the plant dry weight. This fact, together with the appearance of resistance to artemisinin, has led to the development of semisynthetic drugs with improved pharmacological properties such as artemether, arteether, and artesunate (Fig. 14; Brown 2010). Furthermore, *A. annua* is the only commercial source of artemisinin because its chemical synthesis is not commercially feasible and, as a consequence, numerous efforts in metabolic engineering have been made to obtain higher amounts of artemisinin in transgenic plants (Liu et al. 2011). Moreover, the phytotoxicity of artemisinin and two of its biosynthetic precursors (arteannuin B and artemisinic acid) (Fig. 14) has been reported, and they inhibited lettuce seed germination at 33 μM for 24 h. This phytotoxicity was attributed to the peroxide moiety (Duke et al. 1987).

14.2.7 Laurane-Type Carbon Backbone

The genus *Laurencia* (Rhodophyta group) comprises more than 500 different sesquiterpenes (Harizani et al. 2016), and it is one of the most attractive sources of sesquiterpenes among all marine microalgae. The majority of these

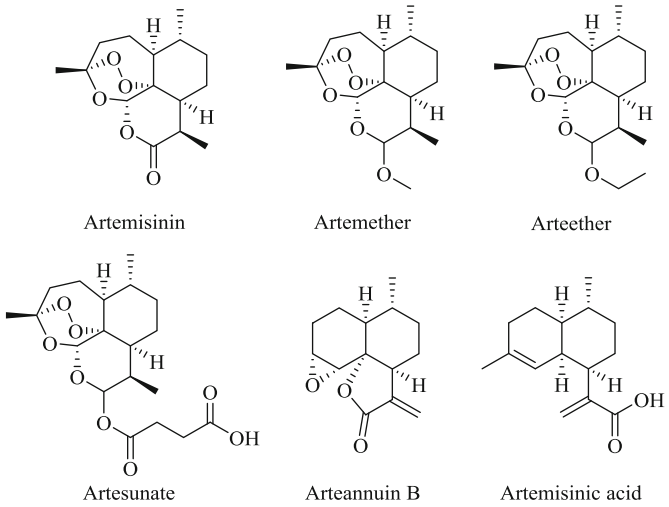
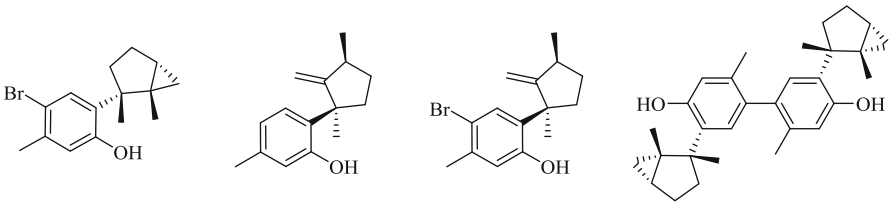


Fig. 14 Semisynthetic drugs with improved pharmacological properties using artemisinin as a lead compound (artemether, arteether, and artesunate) and its biosynthetic precursors (arteannuin B and artemisinic acid)

sesquiterpenes are halogenated, with bromine as the most abundant halogen followed by chlorine. Moreover, other algae also produce brominated sesquiterpenes, including *Neomeris annulata* (Chlorophyta group) (Meyer and Paul 1995; Paul et al. 1993), although in the particular case of Chlorophyta, the sesquiterpenes typically lack the extensive halogenation of the Rhodophyta group (Maschek and Baker 2008). Some halogenated metabolites have shown a wide range of biological activities, including antibacterial, antimalarial, antioxidant, and cytotoxic properties. It has been demonstrated that the nature of the halogen in the structure plays a critical role in the bioactivity (Mao and Guo 2010; Shaza 2014).

Sesquiterpenes with a rearranged laurane-type carbon backbone, which are rare in nature, have been reported and include laurinterol, debromoisolaurinterol, isolaurinterol, or laurebiphenyl (Fig. 15; Mao and Guo 2010). *Laurencia* is generally the main producer of this kind of sesquiterpene. These laurane-backbone sesquiterpenes have shown several beneficial properties, including antimicrobial, insecticidal, and anticancer activities (Ishii et al. 2017; Vairappan et al. 2001). Thus, for instance, laurinterol has shown high antimicrobial activity against strains of *Staphylococcus aureus* (complete inhibition after 48 h at 1–5 μg/mL), *Mycobacterium smegmatis* (1–5 μg/mL), and *C. albicans* (10–100 μg/mL) (Sims et al. 1975). Likewise, miscellaneous biological activities have been reported. Laurinterol and debromolaurinterol possess inhibitory activity against Na^+/K^+-ATPase, with IC_{50} values of 0.04 and 0.4 mM, respectively (Okamoto et al. 2001). On the other hand,



Laurinterol Debromoisolaurinterol Isolaurinterol Laurebiphenyl

Fig. 15 Laurane compounds in algae

moderate cytotoxicity has been observed for laurebiphenyl (found in *L. nidifica* and *L. triticha*) (Harizani et al. 2016) against A-549, BGC-823, BEL-7402, HCT-8, and HeLa cell lines. IC_{50} values of 1.68, 1.22, 1.91, 1.77, and 1.61 μg/mL were obtained, respectively (Jie Sun et al. 2005).

14.2.8 Chamigrane Halosesquiterpenoids

Chamigrane-type backbones (spirane sesquiterpenes) are very common in *Laurencia* spp. Some structures worth highlighting include ma'iliohydrin (Francisco and Erickson 2001), elatol (dos Santos et al. 2010), obtusol, isoobtusol (Martin et al. 1989), yicterpenes A and B, nidificene, and nidifidienol (Fig. 16). As mentioned above, the nature of the halogen in the structure is critical for the biological activity. Li and co-workers evaluated yicterpenes A and B for their inhibition of marine zooplankton (*Artemia salina*), phytoplankton (*Heterosigma akashiwo*), and bacteria (*Vibrio ichthyoenteri*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Bacillus cereus*). The results indicated that the chlorinated yicterpene A was slightly more active against *A. salina*, *H. akashiwo*, and *B. cereus* than the brominated yicterpene B. For instance, LC_{50} values of 219.9 and 305.7 μmol/L after 24 h were obtained for yicterpenes A and B, respectively, against *A. salina* (Li et al. 2013). Furthermore, several studies have shown that these sesquiterpenes play important roles in ecological interactions, such as antiherbivore activity and potential defense against infection by microorganisms, as well as antileishmanial, antibacterial, and cytotoxic activities (Vairappan 2003; Francisco and Erickson 2001).

The chamigranes nidificene and nidifidienol from *L. nidifica* are active against the HSV-1 virus (*herpes simplex* virus type 1) with IC_{50} values of 1.5 and 2.4 μg/mL, respectively (Kimura et al. 1999).

Obtusane (from *L. okamurai*, *L. obtusa*, and other *Laurencia* spp.) showed inhibitory effects on nitric oxide (NO) release from lipopolysaccharide (LPS)-activated RAW 264.7 macrophages ($IC_{50} = 44.9$ μM) and had a slight effect on TNF-

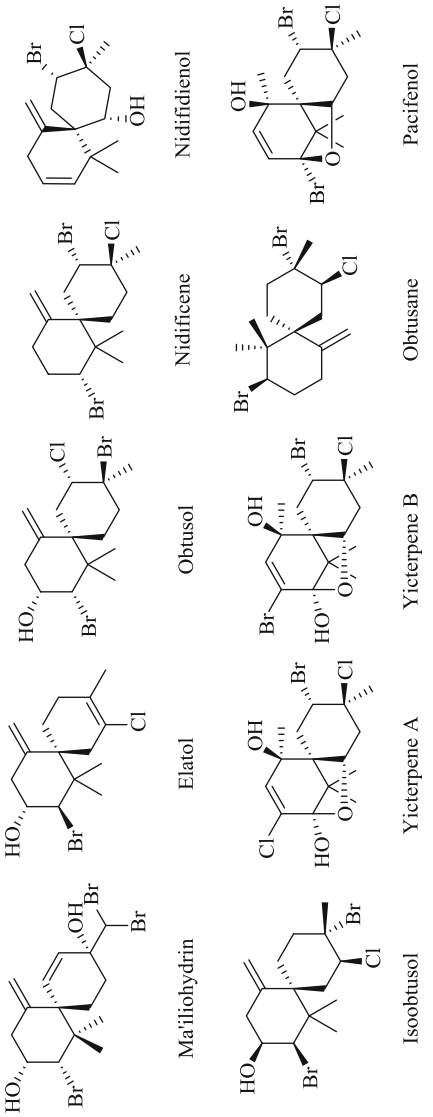
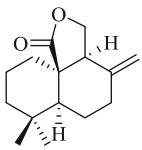


Fig. 16 Chamigrane compounds in algae

Fig. 17 Structure of antrocin



alpha production ($IC_{50} > 250.9$ μM) (Da Silva Machado et al. 2014). This compound was evaluated for antimycobacterial activity against *Mycobacterium bovis* and exhibited an $IC_{50} = 44.7$ μM. This compound showed moderate toxicity in a bioassay of lactate dehydrogenase release with an $IC_{50} = 197.2$ μM. Moreover, pacifenol, which is found in *L. nidifica* and *L. okamurai*, is an effective inhibitor of the degranulation process in human neutrophils, and it inhibits in vitro eicosanoid release stimulated by ionophores. At 100 μM this compound inhibited LTB_4 and TXB_2 release by 82% and 75%, respectively, compared with the control (Gil et al. 1995).

14.2.9 Drimane-Type Sesquiterpenoid

A wide range of natural compounds can be isolated from *A. camphorata*, with terpenoids being the predominant group but with several other types such as sesquiterpenoid, diterpenoid, benzenoid, and benzoquinone derivatives also found. From the sesquiterpenoids present in *A. camphorate*, it is worth highlighting antrocin (Fig. 17), a drimane-type sesquiterpenoid that has proven to show significant activity against different types of cancer (Chiu et al. 2016; Rao et al. 2011). Moreover, extracts from submerged cultivation mycelium of *A. camphorata* produced an anti-inflammatory effect on the murine macrophages cell line RAW 264.7 through inhibition of the expression of iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) (proteins that are responsible of the production of nitric oxide (NO)) and prostangladins, which are important mediators in the process of inflammation (Hseu et al. 2005).

14.3 Bioavailability and Metabolism

14.3.1 Sesquiterpenes with Low Levels of Functionalization

The sesquiterpene α-copaene, which is present in a wide variety of plants, spices, and several citrus fruits, is considered to be essentially insoluble and is not reactive owing to its chemical structure, for which two stereoisomers are described: ylangene and trans-α-copaene. The theoretical log *P* (3.75, from ALOGPS) value indicates a possible localization in cellular membranes and cytoplasm in cells, and this leads to a possible use of α-copaene as a biomarker (Ahmed et al. 2016).

Likewise, valencene is the biological precursor of nootkatone (Sharon-asa et al. 2003), a remarkable sesquiterpene due to its industrial applicability, as described above (Sowden et al. 2005).

Nerolidol is one of the main components of strawberry essential oils. This farnesene-skeleton sesquiterpene can appear in four isomeric forms due to the double bond at C-6 and an enantiomeric carbon at the C-3 position. Several enzymatic complexes could metabolize xenobiotics by oxidation, reduction, hydrolysis, hydration, conjugation, condensation, or isomerization to increase the polarity and excretion. These isozymes are known as P450 or CYP, and they can mainly be found in the endoplasmic reticulum in liver cells because the liver is the first organ involved in drug metabolism. However, a major effect has been observed on CYP located in the small intestine, probably owing to the higher concentration of nerolidol after oral administration (Lněničková et al. 2017).

In a similar way to nerolidol, the chemical structure of farnesol is derived from farnesene. Two isomeric forms (*cis* and *trans*) exist due to the two double bonds, thus *cis*,*cis*-; *trans*,*trans*-; *cis*,*trans*-; and *trans*,*cis*- forms exist. However, the *trans*,*trans* isomeric form is the only one present in all natural sources of this compound. The calculated physicochemical properties show the membrane permeability of this compound because it does not break Lipinski's rule. Although data are not available regarding human bioavailability for farnesol, in vivo studies revealed nontoxic daily doses of around 50 mg (Ku and Lin 2016). Farnesol is an intermediate compound in the cholesterol metabolic pathway in cells. This compound arises from farnesyl diphosphate and is a direct precursor of squalene (Edwards and Ericsson 1999). Furthermore, it is a competitive inhibitor of mevalonate kinase, an intermediate enzyme in the aforementioned biosynthetic pathway. Thus, mevalonate kinase acts as a regulator in lipid metabolism and differentiation (Hinson et al. 1997).

14.3.2 Sesquiterpenes Containing More than One Lactone Ring

Extracts of *G. biloba* leaves are usually formulated as tablets and capsules for oral administration or as an injection for intravenous administration. In an effort to facilitate our understanding of the link between the administration of *G. biloba* leaf extract and its pharmacological action, and to identify the chemical basis of the extract responsible for its therapeutic effects, Liu et al. (Liu et al. 2018) performed rat-based and cell-based pharmacokinetic studies of Yinxing Tongzhi tablets, a standardized *G. biloba* leaf extract, and Shuxuening, a ginkgo preparation for i.v. administration. Bilobalide, the active sesquiterpene in *Ginkgo* extracts, showed an intermediate rate of membrane permeation, it was not affected by the intestinal efflux transporters, and it was poorly excreted into bile in rats. Bilobalide also showed higher oral bioavailability than ginkgolides A and B (active diterpenes also present in *Ginkgo* extracts). Furthermore, bilobalide showed good in vivo reach and limited binding to tissue, it reached both extracellular and intracellular targets, and it was distributed evenly in various body fluids and tissues. Another important

pharmacokinetic characteristic is the elimination route. In the case of bilobalide, glomerular filtration-based renal excretion is the major elimination route, although other elimination routes may be involved, and these require further investigation. Liu et al. concluded that oral administration of Yinxing Tongzhi tablets provided higher levels of systemic exposure than intravenous administration of Shuxuening due to the advantageous pharmacokinetic properties of bilobalide and the low content of bilobalide in the injected dose.

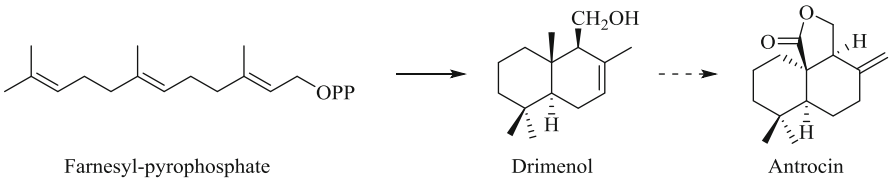
14.3.3 Sesquiterpenes with a 1,2,4-Trioxane Ring

Artemisinin is one of the most active compounds against malarial parasites. However, its low solubility in both oil and water has limited its therapeutic use. Recently, the use of dried leaves of *Artemisia annua* has been proposed as alternative way to supply artemisinin. In a rodent study, dried leaves provided ≈45 times more artemisinin in the serum than that delivered from similar doses of pure artemisinin; they were five times more effective against *Plasmodium chabaudi* and three times more resilient against emerging artemisinin drug resistance. The plant has traditionally been prepared as a tea infusion, but this mode of preparation is not recommended due to the low solubility of artemisinin in water and the low stability of artemisinin at high temperature. Furthermore, the bitter taste of dried leaves and infusions is distasteful for humans. However, this taste could be masked with other foods or by encapsulation (Brown 2010; Ghantous et al. 2010; Kiss et al. 2017).

The bioavailability of artemisinin in this alternative taste masking approach was studied by Desrosiers and Weathers (Desrosiers and Weathers 2016) with artemisinin measured after simulated digestion from dried leaves in four types of capsules in conjunction with protein and protein-based foods: dried milk, casein, bovine serum albumin, peanuts, peanut butter, Plumpy'nut®, and *Artemisia annua* essential oils. Peanuts and Plumpy'nut® led to a decrease in the artemisinin recovered from the liquid digestate fraction. On the other hand, bovine serum albumin led to an increase in artemisinin recovered from liquid simulated digestate fractions, while casein had no effect. The addition of a volume of essential oil equivalent to that found in a high essential oil-producing *A. annua* cultivar also significantly increased the solubility of artemisinin in simulated digestates. Since capsules did not alter the amount of artemisinin extracted from the intestinal liquid, capsules could offer an acceptable alternative to supply *A. annua* dried leaves as a therapeutic source of artemisinin against malaria parasite, and this approach should be tested further in animals and humans.

14.3.4 Laureane and Chamigrane Halosesquiterpenoids

The insertion of halogens such as chlorine and bromine is carried out by haloperoxidases. This seems like a natural response to the abundance of these ions in the marine environment (La Barre et al. 2010). Surprisingly, bromination is more



Farnesyl-pyrophosphate Drimenol Antrocin

Scheme 1 Suggested pathway for the synthesis of antrocin

common than chlorination, even though chlorine is more abundant than bromine in the surroundings. This fact might be due to a bioactivity-related issue, since some brominated compounds seem to be more active as chemical defense agents than those with chlorine.

14.3.5 Drimane-Type Sesquiterpenoids

Antrocin is a sesquiterpene found in *A. camphorata*. To date there is very little available information on how *A. camphorata* synthesizes its metabolites. Lin et al. employed next-generation sequencing (NGS) techniques to study the gene expression of *Antrodia cinnamomea* (Lin et al. 2015), and this served as a first step to provide a better understanding on how terpenoids are synthesized in this species. Although the synthetic pathway of antrocin was not unequivocally elucidated, the authors suggested the pathway shown in Scheme 1 (Lin et al. 2017).

The synthetic route starts with farnesyl pyrophosphate, which is transformed into drimenol by terpene synthases. Different enzymes then modify the structure of drimenol to form a lactone ring through a cyclization.

14.4 Bioactivities (Animal Studies)

14.4.1 Fruits

14.4.1.1 Orange

Valencene is a hydrophobic sesquiterpene with an eremophilane skeleton, and it is found in diverse citrus species. In vivo assays indicate that valencene decreased the melanin content after UVB irradiation in murine B16F10 melanoma. The effect observed was proportional to drug concentration (Nam et al. 2016). These results support the use of this sesquiterpene as a cosmetic against photoaging caused by high skin exposure to UV radiation – the main source of risk of developing skin cancers and precancers.

14.4.1.2 Strawberry

Nerolidol is one of the main components of strawberry essential oils, and a wide range of biological activities have been described for this compound. Growth of

pathogenic or phytopathogenic fungi such as *Candida albicans*, *Microsporum gypseum*, *Fusarium oxysporum*, and *Phytophthora capsici* is susceptible to nerolidol, as supported by numerous in vivo and in vitro studies. This bioactive compound could modify the natural hyphal evolution of the target species (Rahman et al. 2011).

Gastric and duodenal ulcers affect a considerable number of people in the world at least once in their lives. Extrinsic factors such as stress, feeding, smoking, or ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) induce imbalances in the defensive secretions of the mucosa. All treatments inhibit acid secretions by binding to the proton pump in the gastric parietal cell, thus inhibiting the transport of H^+ to the interior of the gastrointestinal tract or by inhibiting the release of histamine in the mast cells of the gastric mucosa, thus increasing the production of gastric mucus. In any case, side effects such as impotence have encouraged the search for alternative therapies. Nerolidol has exhibited significant in vivo reduction in the ulcerative lesion index and a relevant inhibition in the ulcer formation (Klopell and Lemos 2007). Despite the fact that the mechanism of action requires study, nerolidol is a strong candidate as a component in anti-ulcer or gastroprotective therapies.

Likewise, nerolidol or synergistic combinations with linalool, or tea tree oil, showed repellent activity against several species that damage many vegetables and food crops such as maize, beans, potatoes, peppers, tomatoes, and strawberries (Araújo et al. 2012; Kabugi Mwangi et al. 2013).

Additionally, inflammatory response is mediated by a complex system of small molecules and immune cells. The aim of the system is to eliminate or reduce the main cause of tissular or cell damage and repair all implicated tissue. The therapies used are based on analgesics or NSAIDs. However, the duration of these treatments should be short due to their severe side effects, which can include gastrointestinal injures or hemorrhage. The combination of such treatment with any medication must be taken into account. Essential oils from plants that contain a considerable percentage of nerolidol have shown anti-inflammatory effects in mice and rats. Antinociceptive activity has also been described (Fonsêca et al. 2016; Pinheiro et al. 2011).

Finally, in vivo studies have demonstrated potent cytotoxic effects for this sesquiterpene. The weight of tumor in mice injected with melanoma and fed with nerolidol was reduced by a considerable percentage. Moreover, the incidence of intestinal neoplasia in rats was reduced by feeding rats twice per week with nerolidol (Wattenberg 1991).

14.4.1.3 Grapes

Farnesol could be ingested from grapes, and several types of bioactivity such as anti-inflammatory, anti-allergic, antioxidant, chemopreventive, and anticancer have been described. In vivo studies have shown that the administration of farnesol gives rise to anti-allergic and anti-inflammatory effects in asthmatic mice by decreasing immunoglobulin E total levels (Ku and Lin 2015). Moreover, in vitro and in vivo studies have demonstrated that farnesol inhibits cell proliferation and could initiate apoptosis in HeLa, leukemia, hepatoma melanoma, mammary, pancreatic adenocarcinoma,

or lung carcinoma cells. However, human primary T lymphocytes or monocytes have a high resistance to farnesol-induced apoptosis. The mechanisms involved in this different selectivity are unknown, but they could be linked with the antioxidant activity of farnesol. The mechanism of action and pharmacokinetics require further study (Santhanasabapathy and Sudhandiran 2015).

14.4.1.4 Grapefruit

Grapefruit has been associated with antioxidant, anti-inflammatory, and antibacterial activities (Hamdan et al. 2013). The sesquiterpene nootkatone has shown potent in vitro, ex vivo, and in vivo inhibitory effects on platelet aggregation. The benefits observed on cardiovascular disease through coagulation effects make nootkatone a potential therapeutic alternative to commonly used therapies, where limits in use are recommended due to induced adverse effects and resistance.

14.4.2 Vegetables

14.4.2.1 *Curcuma zedoaria* (Christm.) Roscoe

The rhizomes of *Curcuma zedoaria* are used as a stimulant and for stomachic ailments, anti-diarrhea, and wound healing among other benefits in India and Southeast Asian countries, and it is also used in folk medicine in China and Japan. The extract obtained with acetone/H_2O (8:2) from this plant showed a hepatoprotective effect in mice. An in-depth study of the active constituents was performed (Morikawa 2007). Eight sesquiterpenes, including furanodiene, curdione, neocurdione, dehydrocurdione, germacrone, 13-hydroxygermacrone, curcumenol, and zedoarondiol (Fig. 18), may be promising candidates for the effective treatment of lipopolysaccharide-/D-galactosamine (D-GaIN/LPS)-induced acute liver injury (liver-specific toxin). Protective effects against D-GAIN/LPS toxin and against D-

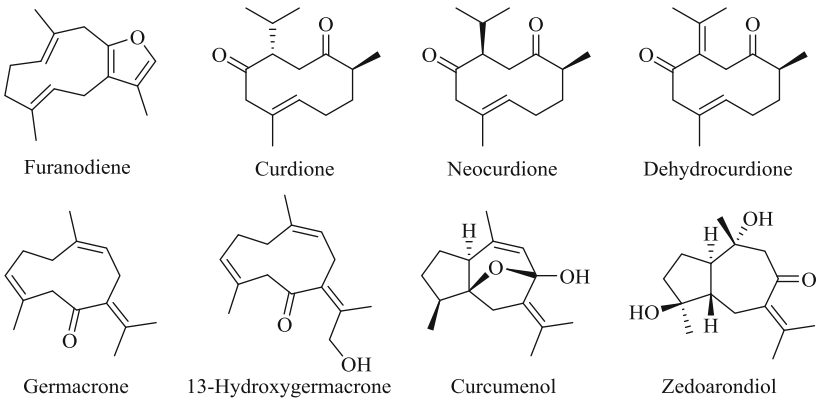


Fig. 18 Active constituents from the rhizomes of *Curcuma zedoaria*

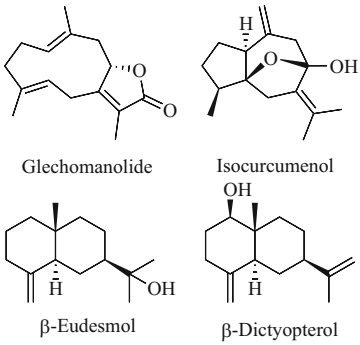


Fig. 19 The most active sesquiterpenes from *Curcuma zedoaria*

GAIN/tumor necrosis factor-α-induced liver injury were observed at 50 mg/Kg upon oral administration. Nevertheless, further studies of the mechanisms of action should be carried out (Morikawa et al. 2002). Likewise, potent vasorelaxant properties have been described for sesquiterpenes derived from this plant. This effect could explain the traditional medicinal value of the rhizomes of *Curcuma zedoaria* for the treatment of "Oketsu" syndrome. Inhibitory effects of the sesquiterpenes on the contractions induced by high K^+ concentration in isolated rat thoracic aorta were evaluated (Matsuda et al. 2001). The following structural requirements were established: the most active compounds were those with germacrane- and eudesmane-type sesquiterpenes; the presence of an *exo*-methylene moiety improved the activity and polyoxygenated sesquiterpenes showed weak activity. The contractions were inhibited by calcium channel-blocking activity. In particular, the most active sesquiterpenes (inhibition >80%, at 100 μM) were germacrone, glechomanolide, isocurcumenol, β-eudesmol, and β-dictyopterol (Fig. 19; Morikawa 2007).

14.4.2.2 *Cichorium intybus* L.

Ether and ethyl acetate extracts of different parts of fresh rhizomes of chicory (hairy roots, inner part, wounded part, neighboring soil, and rhizome skin) were evaluated for their nematicidal activity toward *Globodera rostochiensis* (Nishimura et al. 2000), and the extract from the rhizome skin showed the lowest survival rates with a value of 37.5%.

Two experiments were carried out to study the effect of dietary chicory in cattle against the gastrointestinal nematodes *Ostertagia ostertagi* and *Cooperia oncophora*. In the first experiment, the calves were fed with chicory silage and nutritional complements. In the second experiment, the calves were fed during 7 days with pure forage chicory. *O. ostertagi* counts were reduced by 66% after 22 days on feeding cattle a pure daily dose of chicory (Peña-Espinoza et al. 2016).

Anti-inflammatory effects have been described for chicory extracts. The dried aerial parts of chicory (10 kg) were extracted with ethanol for 1 week. A dose of 500 mg/kg of this extract produced a marked increase in kidney weight when

compared with the control in rats of around 18% and a marked decrease in body weight after the 10th day of treatment (Noori and Mahboob 2009).

14.4.2.3 *Cynara cardunculus* L.

Extracts from artichokes cultivated in Brazil have shown mortality on *Artemia salina.* Artichokes (Noldin et al. 2003) were macerated for 7 days in methanol. The resulting crude product was partitioned in hexane, dichloromethane, ethyl acetate, and butanol. The authors found that the hexane and dichloromethane fractions induced mortality to a significant extent in this shrimp at concentrations of 0.5–2 mg/mL, but the mortality values were not provided. The hexane fraction had an effective concentration of 358 ± 72 μg/mL, which is comparable to that of the positive control quinidine. Although cynaropicrin was the major compound in the dichloromethane fraction, when tested separately, it was not active at 2 mg/mL, thus indicating that another compound could be responsible for the high mortality of microcrustaceans.

Antispasmodic activity has also been reported. In a research project (Emendörfer et al. 2005), the antispasmodic activity of artichoke was evaluated. A crude methanolic extract of leaves was fractionated with hexane, dichloromethane, ethyl acetate, and butanol and then tested for any effect on the guinea pig ileum contractile response elicited by acetylcholine. The ethyl acetate and dichloromethane fractions gave IC_{50} values of 1.22 and 0.93 mg/mL, respectively. The most active fraction from dichloromethane was purified to obtain cynaropicrin. When assayed separately, cynaropicrin had an IC_{50} = 0.065 mg/mL, which is comparable to that of the commercial spasmodic papaverine.

14.4.2.4 Cucurbitaceae Family

Different mixtures of testosterone with pumpkin seed oil (2 and 4 mg/100 g body weight) were administered to rats, and they gave rise to a significant increase in mean prostate size ratio. In this study it was concluded that pumpkin seed oil inhibited testosterone-induced hyperplasia of the rat prostate (Gossell-Williams et al. 2006).

14.4.3 Algae

14.4.3.1 Rhodophyta Algae

The chamigrane elatol (from *Laurencia* spp.) was evaluated in vitro and in vivo for its antitumor properties (Campos et al. 2012). Elatol was able to reduce cell viability with IC_{50} values of 1.1 and 10.1 μM for I.929 and B16F10 cells, respectively. Elatol at a concentration of 50 μM alters the cell cycle progression of B16F10 cells to maintain them in the sub-G_1 phase for up to 48 h in flow cytometry bioassays, and it also causes apoptosis by reduction in the expression of the antiapoptotic protein bcl-xl. Subcutaneous injection in mice of B16F10 cells daily for 10 days caused a

suppression of tumor growth, with a reduction of 71.4% compared with the control and on using the highest tested concentration of 10 mg/kg.

Pacifenol also exerted topical anti-inflammatory effects on the TPA-induced mouse ear edema, with an ID_{50} of 161.1 μg/ear (Gil et al. 1995).

14.4.4 Food with Medicinal Properties

14.4.4.1 Common Butterbur

Butterbur extracts (*Petasites hybridus*) are recommended for the prevention of migraine, and they have been used to treat bronchial asthma and smooth muscle spasms (Schapowal 2002). The pharmacologically active substances of *P. hybridus* are believed to be sesquiterpene esters of the petasin and the furanopetasin chemotype (Chizzola et al. 2006). There is evidence that the extract of the rhizome of *Petasites hybridus* represses leukotriene biosynthesis (Bickel et al. 1994; Thomet et al. 2001), which may be associated with antispasmodic activity and anti-inflammatory action in type I hypersensitivity (Schapowal 2002). This extract also inhibits cyclooxygenase (COX)-2 ($IC_{50} \geq 20$ μg/mL) and COX-1 ($IC_{50} > 400$ μg/mL) activity and ameliorates the activation of p38 mitogen-activated protein kinase stress signaling and IkappaBa in microglial rat cells (Fiebich et al. 2005). Butterbur extracts have also proven to be an effective treatment for hay fever without the sedative effect of the antihistamine cetirizine (Schapowal 2002).

14.4.4.2 *Ginkgo biloba* L.

Standardized *Ginkgo biloba* leaf extract EGb 761® has been used for more than 30 years for cerebral ischemia and dementia due to its neuroprotective effects, as described above. Terpene lactones including ginkgolide B and bilobalide are the most effective constituents. The administration of the sesquiterpene lactone bilobalide (5–20 mg/Kg) injected subcutaneously in mouse model of focal cerebral ischemia demonstrated neuroprotective effects. Administration of this compound 60 min prior to ischemia reduced in a dose-dependent manner the infarct area in mouse brain and in rat brain 2 days after the onset of the injury (Ahlemeyer and Krieglstein 2003). It has also been demonstrated that bilobalide allows mitochondria to maintain their respiratory activity in ischemic conditions by protecting complex I and probably complex III activities. An increase in the respiratory control ratio (RCR) of cerebral mitochondria by 26 and 24% at 8 and 10 mg/Kg, respectively, has been reported by Janssens and co-workers (Janssens et al. 2000).

14.4.4.3 *Chrysanthemum parthenium* (L.) Bernh.

The effects of a chloroform extract of fresh leaves of the herb feverfew on potassium currents in smooth muscle were studied by Barsby et al. (1993b). The extract reduced the inactivating voltage-dependent potassium current in a concentration-related manner, with an IC_{50} value of 56 μg mL^{-1}. Also, feverfew decreased the time

to peak of the current and increased the rate of decay of the current. These results suggest that chloroform extracts of feverfew leaf are capable of producing a selective, open-channel blocking of voltage-dependent potassium channels (Barsby et al. 1993b).

Parthenolide is the main component obtained from the leaves of feverfew (*Tanacetum parthenium*). This plant has been used orally or as an infusion to treat migraine, arthritis, fever, and stomachache. The chemical characteristics of parthenolide (α-methylene-γ-lactone ring and epoxide molecule) enable this moiety to interact with nucleophilic sites of biological molecules. Besides its anti-inflammatory and anti-migraine properties, this sesquiterpene lactone has also shown anticancer effects. An in vivo animal model study carried out by Won et al. demonstrated that this natural product led to a remarkable reduction in the incidence and multiplicity of papilloma. Female SKH-1 hairless mice were employed in this experiment, and they were fed with fresh food pellets containing parthenolide three times a week (1 mg parthenolide/day) for 25 weeks. The results showed a chemopreventive property of parthenolide against UVB-induced skin cancer in mice. The onset of tumor incidence was delayed from week 13 to week 18, the multiplicity of papilloma was reduced by 30%, and a decrease in tumor size was observed (Won et al. 2004).

14.4.4.4 *Taraxacum officinale* (L.) Weber ex F. H. Wigg.

Dandelion is a wild plant that is used as a human food and in folk medicine. Several in vivo studies have been performed on rabbits and mice to evaluate the acute toxicity of this plant. Rabbits were orally administered with dried dandelion plant up to 6 g/Kg body weight for 7 days, while mice were treated with an ethanolic extract, and significant effects or visible toxicity were not observed (Wirngo et al. 2016).

14.4.5 Mushrooms

14.4.5.1 *Antrodia Camphorata*

A. camphorata has traditionally been used as a medicine to protect liver cells. In a study with rats (Dai et al. 2003), oral administration of the mycelia and sporocarps of *A. camphorata* proved to protect the liver against damage induced by ethanol. When rats were administered with ethanol, an increase in the levels of glutamate-pyruvate aminotransferase (GPT) and glutamate-oxaloacetate aminotransferase (GOT) in serum and an increment of the activity of hepatic superoxide dismutase (SOD) were observed. These signals provide proof that hepatic cells are damaged. When liver cells are damaged, the GPT and GOT in the liver are released to serum. SOD is one of the major antioxidant enzymes in liver, and it has been found that damage to liver cells induces the expression of SOD (Zhao et al. 1996). When the rats were administered *A. camphorata* mycelia or sporocarps, the serum levels of GPT and

GOT decreased, and the concentration of SOD was lower than in the control rats, which were only given ethanol. It is suggested that the liver protective activity is associated with the antioxidant activity.

14.5 Benefits (Human Studies)

14.5.1 Fruits

14.5.1.1 Strawberry

Skin irritation and sensitization studies are commonly carried out after occlusive drug applications during 48 h. Edema and erythema were cleared if a negative reaction had occurred after 48 h. Human studies have revealed that nerolidol (4%) in petrolatum causes a negative response after a patch test reaction (Lapczynski et al. 2008). Moreover, a combination of nerolidol and tea tree oil provides an effective alternative for treating pediculosis, an infestation that is now extensive in undeveloped and developed countries (Di Campli et al. 2012).

14.5.2 Food with Medicinal Properties

Artemisinin, parthenolide, and some of their derivatives are lead compounds in clinical trials due to their selectivity toward tumors and cancer stem cells. For example, artemisinin-derived drugs are promising for laryngeal carcinomas (Singh and Verma 2002), uveal melanomas (Berger et al. 2005), and pituitary macroadenomas (Singh and Panwar 2006) and are in phase I–II trials against lupus nephritis and breast, colorectal, and non-small cell lung cancers (Lu 2002; Zhang et al. 2008). Dimethylamino parthenolide is currently in phase I against acute myeloid leukemia, acute lymphoblastic leukemia, and other blood and lymph node cancers (Guzman et al. 2007). One of the most promising characteristics of SLs is their selectivity to target tumor and cancer stem cells while sparing normal ones (Kawasaki et al. 2009). The selectivity shown by sesquiterpene lactones is attributed to their ability to target the sarco-/endoplasmic reticulum calcium ATPase (SERCA) pump, specific proteases secreted by cancer cells, high iron content and cell surface transferrin receptors, nuclear factor-kB (NF-kB) signaling, MDM2 degradation and p53 activation, angiogenesis, metastasis, and epigenetic mechanisms (Ghantous et al. 2010). However, the low amount of these compounds produced by natural sources and their poor bioavailability, in some cases owing to their extensive plasma protein interactions and hydrophobicity, have made their commercialization difficult. Furthermore, it is necessary improve their toxicological profiles, allergenic properties, and off-target effects (Ghantous et al. 2010).

The anticancer activity of the aforementioned compounds is usually attributed to the exocyclic α-methylene-γ-lactone moiety, which can react by Michael-type

addition with biological nucleophiles to form stable adducts. However, this reactivity is also responsible for reducing the bioavailability of SLs because they bind to blood proteins that contain sulfhydryl groups, thus preventing them from achieving concentrations that are crucial to cytotoxicity (Wagner et al. 2004). More recent studies have provided evidence that the number and type of alkylating centers (α-methylene-γ-lactone, α-methylene-δ-lactone, conjugated cyclopentenone, and conjugated side chain ester) are very important for antitumor activities (Maries et al. 1995; Schmidt 1999; Schmidt et al. 1999; Siedle et al. 2003). For example, the presence of the unsaturated carbonyl systems, whether in lactone or cyclopentenone rings or in an ester side chain, increases SL toxicity toward tumor cells. In the cases of carcinosarcomas and chronic adjuvant arthritis, in addition to the α-methylene-γ-lactone and the β-unsubstituted cyclopentenone, it is also a prerequisite to have an α-epoxycyclopentanone for antitumorigenic and anti-inflammatory properties (Ghantous et al. 2010).

Two other important factors involved in the cytotoxic activity of SLs are lipophilicity and molecular geometry. The lipophilicity can be modified by changes in the side chains, usually as ester groups. In general, higher lipophilicity can facilitate penetration through the cell membrane, but this can also decrease beyond a certain size limit, causing steric hindrance on the exocyclic methylene group and preventing the appropriate approach to its target (Guzman et al. 2007). The presence of hydroxyl and methoxyl groups in SLs and their influence on cytotoxicity is not clear, and contradictory results have been published (Bruno et al. 2005; Ghantous et al. 2009; Schmidt and Heilmann 2002). On the other hand, flexible SLs have shown higher cytotoxic activity; for instance, cis-fused lactone rings are more flexible than trans-fused systems, and they have shown higher activity (Beekman et al. 1997; Schmidt et al. 1999). Stereochemistry has also been reported to be an important factor in the anticancer activity of SLs. For instance, parthenin (β-OH isomer at C-1) is more active against ethyl phenylpropiolate-induced mouse ear edema than hymenin (α-OH isomer at C1) (Ghantous et al. 2010).

14.5.2.1 *Ginkgo biloba* L.

Ginkgo biloba extracts have become one of the most popular dietary supplements in America due to their positive effect on memory, their improvement in cognitive function, and their potential beneficial effects in patients with Alzheimer's disease. Janßen et al. reviewed the articles concerning the use of *G. biloba* extracts in the treatment of Alzheimer's disease. It was established that a high dose of *Ginkgo* (240 mg) has a beneficial effect for the cognition outcomes and accompanying psychopathological symptoms. However, data regarding the risk and side effects are still needed as well as a comparison of the benefits in relation to other drugs approved for Alzheimer's disease. For these reasons, long-term studies are necessary to assess the beneficial and adverse effects of long-term therapy with *Ginkgo* (Janßen et al. 2010). Moreover, the standardized leaf extract of *Ginkgo* (EGb 761®) has been used in clinical trials for the treatment of vascular and cognitive disorders. These

extracts contain 24% flavonoids and around 6% terpenoids (bilobalide and ginkgolides among others). The improvement in cognition effects is attributed to the sesquiterpenoid bilobalide, which acts on hippocampal pyramidal neuronal excitability. This sesquiterpenoid acts as an antagonist on the main inhibitory neurotransmitter in the central nervous system (γ-aminobutyric acid (GABA) receptors) acting in the chloride channels (Johnston et al. 2009). Furthermore, a double-blind placebo-controlled pilot study of a standardized *Ginkgo biloba* extract (Seredrin) was performed on patients with primary Raynaud's disease (RD). RD is a common and painful condition characterized by episodic digit ischemia produced by emotion and cold. The treatments available produce high levels of side effects and new alternatives are needed. A total of 22 patients with RD were enrolled in this study, with half receiving a total of 360 mg *Ginkgo biloba* extract per day (divided into three doses of 120 mg) and the other half a matching placebo with a total treatment duration of 10 weeks. A reduction in the number of attacks per day of 56% was observed in the group treated with Seredrin, whereas a reduction of 27% was noted with the placebo. Moreover, significant differences in hemorheology were not observed between the two groups. These preliminary results indicate that *Ginkgo biloba* extract showed positive trends for the treatment of RD. However, in order to confirm its potential, more studies with a larger number of patients should be conducted (Muir et al. 2002).

14.5.2.2 *Artemisia annua* L.

Clinical studies in the late 1970s with patients infected with *P. vivax* or *P. falciparum* demonstrated that artemisinin could kill the malarial parasite, and it was even shown to be completely effective in the treatment of chloroquine-resistant *Falciparum malaria* without obvious side effects. However, the low solubility of artemisinin in both water and oil has limited its therapeutic use. In an effort to improve the pharmacological properties of artemisinin, several semisynthetic derivatives have been tested, with artemether, arteether, and artesunate being the most important, and these showed improved activity when compared to artemisinin and had higher solubility and metabolic and hydrolytic stabilities (Brown 2010).

14.5.2.3 *Taraxacum officinale* (L.) Weber ex F. H. Wigg.

Dandelion is generally considered to be safe, with rare side effects, and it also has been reported to be a weak sensitizer, and allergic reactions have not been ruled out in sensitive people. The allergen in this plant has been identified as taraxinic acid 1′-*O*-β-glucopyranoside. An in vivo study with 235 adults divided into 4 groups (patients with allergic contact dermatitis, patients with atopic dermatitis, nonatopic patients suffering from non-allergic chronic inflammatory illnesses and healthy volunteers) was performed by Jonanovic et al. to evaluate the response of patch testing supplemented with dandelion extract. The results showed that allergic reactions were obtained only in patients with eczema, and irritant reactions were not recorded for dandelion extract. These outcomes reinforce the safety of dandelion consumption (Jovanovic et al. 2004).

14.6 Applications in food

14.6.1 Fruits

14.6.1.1 Apple

α-Farnesene can be used as an artificial flavoring in sour or tart fruits such as kiwi, carambola (star fruit), and green apple.

14.6.1.2 Orange

δ-Cadinene can be used as a flavoring ingredient because the substance, extract, or preparation imparts an agreeable or attractive smell. Likewise, valencene is currently used as a fragrance and flavor in beverages and in chewing gum in particular.

14.6.1.3 Strawberry

Nerolidol is a common flavor intensifier that is widely used in the food industry since it was approved by the US Food and Drug Administration (Weng-Keong et al. 2016).

14.6.1.4 Grapes

The American Food Drug Administration considers farnesol apt for human consumption; however, doses are restricted. They are used in the minimum quantity required to produce their intended effect and otherwise in accordance with all the principles of good manufacturing practice.

14.6.1.5 Grapefruit

Nootkatone is used as a fragrance and as a food additive flavoring agent.

14.6.2 Vegetables

14.6.2.1 *Cichorium intybus* L.

Chicory is an important agricultural crop because its roots and leaves have several uses. For instance, the roots are cooked as a vegetable or roasted as a coffee surrogate, and they are an excellent source of inulin or fructose. The leaves are consumed as a vegetable and are used as livestock forage, especially for ruminants, and the sprouts are consumed raw as admixtures to salads (Barry 1998; Wang and Cui 2011). The bitter taste is generally disliked by consumers, and this is the main reason for the rejection of several foods. Different strategies have been adopted to reduce the content of SLs in chicory. One of these strategies is the breeding of cultivars with very low levels of SLs, but the low content of SLs reduces the resistance against disease or herbivore attack, thus leading to lower crop yields or the increased application of pesticides. Cut chicory was traditionally soaked in cold or warm water to debitter prior to consumption. Wulfkuehler et al. demonstrated that SL levels in freshly cut chicory were markedly lowered by applying warm water (45 °C, 120 s), with SL levels reduced by 60.9–64.5%, and concomitantly the sensory quality and shelf life were improved (Wulfkuehler et al. 2013). However,

the wounds and decompartmentalization of plant cells produced by shredding of freshly cut chicory led to undesired browning, which is catalyzed by oxidative enzymes such as polyphenol oxidase. This browning facilitates microbial growth and accelerates spoilage. In contrast to blade cutting, high-pressure water-jet cutting provides a sterile and permanently renewing cutting medium, thus completely preventing cross-contamination. Wulfkuehler et al. demonstrated that water-jet cutting did not affect the physiological condition, the microbiological quality, or the levels of SLs in freshly cut radicchio when compared to blade cutting using a newly sharpened blade (Wulfkuehler et al. 2015).

14.6.3 Algae

Algae are 80–90% water, and, after drying, they contain 50% carbohydrates, 1–3% lipids, 7–38% minerals, and a highly variable content of proteins (10–47%). The nutritional content varies not only between species but also depending on the date of collection of the algae. For example, *Ulva* sp. contains 57.5 ± 31.1 mg iron/100 g dry weight and 232.28 ± 48.84 mg ascorbic acid/100 g dry weight (Garcia-Casal et al. 2007). However, the selection of edible species with commercial value is based on texture and taste rather than nutritional value (Pereira 2016).

Methods for preparing dishes with algae vary between different countries. In Japan algae are served cold after brief blanching, and different condiments are added such as vinegar, soy sauce, salt, or sugar, while in China they are cooked with pork or noodles and eaten hot (Bangmei and Abbott 1987). In general, algae are consumed with little or no cooking as an accompaniment to raw fish. *Caulerpa* spp. have peppery tastes of different intensities depending on the species (Higa and Kuniyoshi 2000; Vidal et al. 1984), and they can be used for making salads and sauces (Pereira 2015).

14.6.3.1 Chlorophyta Algae

Ulva spp. (Chlorophyta group) that have been studied for their application in the food industry include *U. lactuca*, *U. pertusa*, *U. compressa*, and *U. clathrata*. These are consumed as food condiments and as nutritional supplements in Japan and China. For example, in Japan they are used in salads, soups, cooked meals, and condiments or mixed with other green seaweeds. They are not as widely used in Western countries, but they have been authorized for human consumption in France (Raj et al. 2016).

In order to preserve the phytochemicals, *Ulva* spp. can be dried in a microwave with a very low power (300 W) for 3 min.

14.6.3.2 Cyanophyta Algae

Arthrospira platensis (Cyanophyta) is an edible alga that can be used as an ingredient in flours to add consistency, and it is a source of vitamins and proteins (Zouari et al. 2011).

14.7 Safety, Toxicity, and Side Effects

14.7.1 Fruits

Hazards have not been detected by the European Food Safety Authority for α-farnesene (apple), δ-cadinene (orange), or farnesol (grape).

14.7.1.1 Orange

α-Copaene has been described as being flammable and irritating to skin. During manipulation this compound should be kept out of the reach of children, and skin contact should be avoided by wearing suitable gloves. Moreover, for the sesquiterpene valencene, inhalation or aspiration should be avoided because it could cause lung damage. Otherwise, despite the consumption of fruits and processed foods that contain α-ylangene, its use as flavor or fragrance is not recommended.

14.7.1.2 Strawberry

Safety data for nerolidol have been published. LD_{50} values for oral or parenteral toxicity relative to rat or rabbit are higher than 5000 mg/Kg. In humans tests on a 4% solution of nerolidol did not indicate any irritation or sensitization.

14.7.1.3 Grapefruit

Nootkatone or (+)-nootkatone, the most important and expensive sesquiterpene in grapefruit, is used as a fragrance ingredient, and this should be at least 98% pure, with a melting point of at least 32 °C. Lower purity grades cannot be used as a fragrance ingredient.

14.7.2 Vegetables

14.7.2.1 *Cichorium intybus* L.

Although chicory has been part of the human diet for a long time, the sesquiterpene lactones contained in it might be toxic in large amounts. A 2007 study concerning different toxicological bioassays on the sesquiterpene lactones from chicory roots concluded that chicory root extracts are safe for human consumption and may be used as therapeutic agents (Schmidt et al. 2007).

14.7.2.2 *Cynara cardunculus* L.

Dermatitis cases in humans have been reported after handling artichoke, and it is associated with the presence of cynaropicrin and other related sesquiterpene lactones in this vegetable. A report from 1983 (Meding 1983) indicated that a 44-year-old man with no previous history of allergies had an allergic reaction to eating and handling artichokes, with positive results obtained after 72 h in patch tests carried out on this man using the stem of an artichoke and an ethanol extract of the stem. A recent review (Paulsen 2017), which lists a series of reports of allergic dermatitis suffered after exposure to different sesquiterpene lactones contained in vegetables,

confirms the suspicion that there is a risk of experiencing different allergic reactions such as eruptions on the skin or even respiratory problems. Lettuce, artichoke, and chicory are among the vegetables with reported cases of allergies.

14.7.3 Algae

A remarkable phenomenon related to toxicity in algae is called "red tides" or "harmful algal blooms," when the population of microscopic algae grows by massive amounts. One of the forms of these phenomena is the release of harmful toxins to the water, and these affect fish and other marine organisms (Anderson 1995). *Gymnodinium nagasakiense* is a dynophyte microalga that blooms near Japan (Kajiwara et al. 1992) and produces compounds that alter the growth of other members of the phytoplankton. One of these compounds is the sesquiterpene cubenol, which showed cell-destroying activity at 5 ppm after 30 min against the species *Heterosigma akashiwo*, *Chattonella marina*, *C. antiqua*, and *G. nagasakiense*. This compound is also present in the marine brown algae *Dictyopteris divaricata* and *Undaria pinnatifida*.

14.7.3.1 Chlorophyta Algae

Caulerpa spp. also contain other compounds, including a red-colored alkaloid named caulerpin and caulerpicin, a mixture of sphinganine-derived ceramides (Higa and Kuniyoshi 2000; Nielsen et al. 1982). Together, these compounds have been associated with the toxicity of *Caulerpa* spp., but Vidal et al. indicated that caulerpin and caulerpicin were not responsible for the toxicity of *C. scalpelliformis* (Vidal et al. 1984) because none of the mice died after oral or intraperitoneal administration of up to 2 g/kg or intravenous injection of 0.2 g/kg of caulerpin (Higa and Kuniyoshi 2000).

14.7.4 Food with Medicinal Properties

14.7.4.1 Common Butterbur

Common butterbur, in addition to the beneficial sesquiterpene lactones, contains pyrrolizidine alkaloids (PAs) that are hepatotoxins in animals and humans (Stegelmeier et al. 1999) and have shown carcinogenic and mutagenic potential (Roeder 1995). The highest concentrations are often located in the rhizomes and stalks, with the lowest concentrations in the leaves. The relative concentrations can change depending on where the plants are grown. Therefore, plants intended for use as pharmaceutical ingredients should be low in PAs, and the German Federal Health Bureau established regulations that restrict the oral exposure to pyrrolizidine alkaloids or their *N*-oxides in herbal preparations to 1.0 μg/day (intake restricted to 6 weeks only) or 0.1 μg/day (no restrictions on intake) with the exclusion of pregnant and lactating women, for whom zero exposure is recommended (Stegelmeier et al.

1999). Avula et al. (2012) demonstrated that 7 of 21 dietary supplements analyzed and two species of *Petasites* (*P. hybridus* and *P. frigidus*) contain PAs.

14.7.4.2 *Ginkgo biloba* L.

Although there is a need to study in more depth the risks that *Ginkgo biloba* extract consumption could have, some adverse effects have been reported, including gastrointestinal symptoms, headache, nausea, and allergic skin reactions. Likewise, adverse interactions with common prescription medications have been described (Gornik and Creager 2013). Bleeding reactions have been associated with the combination of *Ginkgo* with aspirin, rofecoxib, or warfarin, and coma has been reported in a patient with Alzheimer's disease who took *Ginkgo* leaf with trazodone (an antidepressant drug) (De Smet 2002).

14.7.4.3 *Ambrosia artemisiifolia* L.

The herb of *A. artemisiifolia* has gained popularity as a medicinal plant and food. Several products are available on the market, and these are typically food supplements (dry ragweed powder, alcoholic extract) or foods (puree made from the fresh buds of the plant). However, the widespread and long-standing folk medicinal application of this plant is not supported by the available data. Furthermore, there are no human studies to support these therapeutic indications, and it is necessary to establish the safety of this herb for human use. With this aim in mind, Kiss et al. studied the repeated dose toxicity of a product containing puree of ragweed herb in a rat model. The results showed some protective effects on the liver and on triglyceride levels during oral ragweed consumption. However, significant changes in the carbamide level and relative organ weights were also found, and these changes are probably indicative of toxic effects on the kidney and brain.

Sesquiterpene lactones may also play a role in some beneficial effects of ragweed, but, on the other hand, these compounds may also have cytotoxic effects. In view of their results, Kiss et al. concluded that the consumption of ragweed is questionable since there are no human toxicological studies, and the results of their animal experiment should be considered as a warning signal (Kiss et al. 2017).

14.7.4.4 *Taraxacum officinale* (L.) Weber ex F. H. Wigg.

Dandelion is used as a salad ingredient, and its roots and extracts are used as a coffee substitute. Rare side effects have been described for this weed, although contact dermatitis, diarrhea, and gastrointestinal upset have been reported. Generally, this plant is not recommended for patients with liver or gallbladder ailments as it has been postulated that dandelion-stimulated bile secretion may occur, although there is no direct scientific evidence for this (Sweeney et al. 2005). The recommended doses for the consumption of this plant have been established by the European Commission and the *British Herbal Pharmacopoeia*. These daily dose recommendations are as follows: fresh or dried leaves (4–10 g), fresh leaf juice (1 teaspoon twice), fluid extract (1–2 teaspoon), fresh roots (2–8 g), and dried powder extract (250–1000 mg four times) (Wirngo et al. 2016). Likewise, dandelion is recognized as a safe food in

the USA with a maximum level of 0.014% for fluid extract and 0.003% for solid extract (Sweeney et al. 2005).

14.8 Marketed Products

14.8.1 Fruits

14.8.1.1 Apple

α-Farnesene is used as a fragrance in rose and other floral compositions. Furthermore, it is used in cosmetics and in perfumes.

14.8.1.2 Orange

There are a number of companies that supply valencene with different levels of purity. Besides, α-ylangene is used for the preparation of fruits, ice creams, yoghurts, and beverages.

14.8.1.3 Strawberry

Nerolidol is frequently found in cosmetics, shampoo, body lotions, and perfumes and as an aroma component of household cleaners and detergents. It has been estimated that the worldwide consumption of nerolidol is in the range from 10 to 100 metric tons (Lapczynski et al. 2008).

14.8.1.4 Grapes

Farnesol is an aromatic constituent in thousands of products such as cosmetics and personal care products, aftershave lotions, cleansing products, colognes, deodorants, eye lotions, face powders, foot powders, fragrances, hair care products, moisturizers, shaving products, and skin care products.

14.8.1.5 Grapefruit

A study of several commercial aromatized vinegars has shown that nootkatone is present in lemon aromatized vinegar, and this imparts its antioxidant activity (Cejudo-bastante et al. 2012).

14.8.2 Vegetables

14.8.2.1 *Cichorium intybus* L.

The commercial part of the chicory plant comprises mainly the innermost leaves, while the outermost leaves are typically removed, and the roots are considered to be waste (Poli et al. 2002). However, chicory roots can be dried and roasted to prepare infusions as coffee substitutes (Schmidt et al. 2007).

14.8.2.2 *Cynara cardunculus* L.

Artichoke leaf extracts are sold as nutraceutical products in pills by different companies, and these can be easily purchased from well-known online stores. Commercial artichoke products include detox preparations in powder (e.g., from Karéléa™ or Artichoke Romarin from Laboratoires Vitarmonyl©), artichoke extracts (Gerlinéa™), drinks to improve digestion in which artichoke is combined with other ingredients such as green tea (AquaLigne™), and artichoke root extracts (Laboratoires Juvamine©), among others.

14.8.2.3 Cucurbitaceae Family

Pumpkin seed oil is sold in pharmacies in the form of a capsule manufactured by the company Good 'N Natural, with each capsule containing 1000 mg of *C. pepo* seed oil (Gossell-Williams et al. 2006). It has been reported that this oil is useful for the treatment of urinary problems related with benign prostatic hyperplasia.

14.8.3 Algae

More than 200 species are processed commercially on the coasts of Korea, Japan, China, the Philippines, Taiwan, Indonesia, Russia, Italy, France, the USA, and Chile. About 60–70% of the 16 million tons of seaweed produced per year was used for human consumption in 2005 (Hudek et al. 2014). Furthermore, the company ALGA^{+} dedicates its commercial activity to the cultivation of macroalgae in Europe (www.algaplus.pt).

14.8.3.1 Cyanophyta Algae

Spirulina is a dietetic supplement obtained from the microalgae of the genus *Arthrospira*. This product is sold under the name spirulina by several companies such as Aldous Labs, Ibornatur, and Nutrex.

14.8.4 Food with Medicinal Properties

14.8.4.1 Common Butterbur

Butterbur extracts are sold by many companies, including Now, Solaray, and Swanson, as different formulations, e.g., capsules, tablets, or softgels. The formulation and the extraction procedure can influence the content of both the beneficial petasins and dangerous PAs, and, for this reason, it is extremely important to know the concentration of these compounds to ensure the safety of the products. Avula et al. (2012) analyzed 21 commercially available petasin products, and they demonstrated that only 7 contained the amount of petasins stated on the package and 7 contained PAs (Avula et al. 2012). For these reasons, more control and better regulation are required to ensure the safety of commercial butterbur extracts.

14.8.4.2 *Ginkgo biloba* L.

Ginkgo biloba extracts are commercialized as tablets, capsules, or softgels by several companies. The standardized commercial extracts of *G. biloba* leaf used in ginkgo supplements contain no less than 6% sesquiterpene lactones and 24% flavonol glycosides, since this concentration has been extensively studied in human clinical trials. While sesquiterpene lactones are only found in ginkgo leaf, the flavonol glycosides are present in many other botanical extracts. However, due to the high production cost and high demand, there is an incentive to adulterate ginkgo extracts with pure flavonols (e.g., rutin, quercetin, kaempferol) or flavonoid-rich materials from less expensive sources to achieve the desired 24% flavonol glycoside content (Avula et al. 2015). The *United States Pharmacopeia 37-National Formulary 32* (USP37-NF32) specified that powdered ginkgo extract should have a flavonoid content of 22–27%, calculated as flavonol glycosides, and a maximum content of 5 ppm of ginkgolic acids, which are known contact allergens. In addition, specifications are also provided for the terpene lactones (bilobalide and ginkgolide A, B, and C), i.e., not less than (NLT) 5.4% and not more than (NMT) 12.0% (Pharmacopeia 2014). Avula et al. (2015) analyzed 25 commercially available *G. biloba* dietary supplements, and 11 of them showed clear evidence of adulteration with *S. japonicum* fruit or flower.

14.8.4.3 *Chrysanthemum parthenium* (L.) Bernh.

In the case of feverfew, preparations of fresh or dried leaves are widely consumed in the UK as a remedy for arthritis and migraine, but the pharmacological basis for this has not been established. Barsby et al. (1993b) determined that there are significant differences in the pharmacology of chloroform extracts of fresh feverfew leaves versus commercially available powdered leaves. For example, the fresh leaves contain an inhibitory principle(s) that causes a time-dependent, non-specific, and irreversible inhibition of the action of smooth muscle agonists and also causes a slow loss of tone of precontracted muscle. In contrast, extracts of powdered leaves did not exert inhibitory effects on the smooth muscle preparations. This difference could be due to the absence of parthenolide and other sesquiterpene lactones in the powdered leaves (Barsby et al. 1993a).

14.8.4.4 *Litsea cubeba* (Lour.) Pers.

Essential oil from the fruits of *Litsea cubeba* L. is rich in citral (around 70%), and prominent bioactivities have been reported. This oil has a very aromatic and lemonlike spicy odor, and it has been approved by the Ministry of Health in China as a food additive in accordance with normative GB 2790–2007 (Si et al. 2012). Commercial essential oils from *Litsea cubeba* L. are marketed under the names May Chang oil and Kangrean oil (from Central Java, Indonesia) (van Hulssen and Koolhaas 2010).

14.8.5 Mushrooms

14.8.5.1 *Antrodia camphorata*

Extracts of *A. camphorata* are sold as functional foods and dietary supplements or as drinks and capsules, mainly due to their hepatoprotective, anticancer, and antioxidant activity. Additionally, *A. camphorata* mycelia extract that is rich in polysaccharides is sold among other fungi extracts as a "mushroom drink," which is a dietary supplement. In many other cases, the extract of this *Antrodia* species is sold as capsules.

14.9 Patents

14.9.1 Fruits

14.9.1.1 Strawberry

A process to retrieve larger quantities of nerolidol from essential oils has been patented. The extraction method of the invention allows a reduction in the loss of volatile oils to give an improved yield of 35% or more and a high active ingredient content – in particular the trans-nerolidol content was significantly increased (Meng et al. 2018).

14.9.1.2 Grapes

Several inventions related to farnesol have been published, including the synthesis of *trans*,*trans*-farnesol (Zeng et al. 2016), the identification of the safe and effective amount of farnesol for the regulation of mammalian keratinous tissue (Lynn Bissett and Jewell-Motz 1999), and its use as a component of an antiplaque oral composition (Nabi et al. 1995).

14.9.1.3 Grapefruit

Diverse patents have been submitted in the past year for nootkatone. These include a composition that provides the release of fragrance over an extended period of time (Goldblum and Warren 2014).

14.9.2 Vegetables

14.9.2.1 *Cichorium intybus* L.

In an effort to reduce the bitterness of chicory plant material or flour, (Peet and Justice 2015) described an invention to make low-bitterness chicory chips and flour food products such as dairy products, yoghurts, ice creams, milk-based drinks, milk-based garnishes, puddings, milkshakes, egg custard, cheeses, nutrition bars, energy bars, breakfast bars, confectionery, bakery products, crackers, cookies, biscuits, cereal chips, snack products, ice tea, fruit juice, diet drinks, sodas, sports drinks, powdered drink mixtures for dietary supplementation, infant and baby food, calcium-

supplemented orange juice, bread, croissants, breakfast cereals, pasta, noodles, spreads, sugar-free biscuits and chocolates, calcium chews, meat products, mayonnaise, salad dressings, nut butter, sauces, and soups. Presently, the removal of the bitter taste is a costly step in the production of inulin from chicory roots, which makes chicory fibers significantly more expensive than cellulose fibers. For these purposes, they considered how chicory plants are cultivated; the procedure for cooking chicory taproots; the drying process of the chicory taproots after cooking to reduce moisture; the concentration of dihydrolactucin, dihydro-8-deoxy-lactucin, lactucin, 8-deoxylactucin, and lactucopicrin; and the milling of chicory taproots (Peet and Justice 2015).

Compositions that can be used to enhance human or animal health and to prevent or treat inflammation that include one or more phytochemical agents derived from a plant source such as chicory are described in another patent (Malnoe et al. 2004). The inventors describe how a chicory extract made with ethyl acetate had a pronounced effect on the inhibition of cyclooxygenase activity by causing a decrease in the amount of PGE2 (95% reduction at 100 μg/mL dose compared with the control).

14.9.2.2 Cyperaceae Family

A patent was filed on the use of extracts of *Cyperus rotundus* in the treatment and prevention of menopausal diseases caused by the decline in estrogen levels or by allergic diseases (Kwak et al. 2015). This patent describes the use of extracts of *C. rotundus* or a series of six sesquiterpenes with high estrogenic activity. The structures of these compounds are provided in Fig. 20. In the patent it is explained that 4α,5α-oxidoeudesm-11-en-3-one, cyper-11-ene-3,4-dione and 3α-methoxyeudesma-4,11-diene showed high estrogenic activity and markedly reduced activities by co-treatment with tamoxifen to work as estrogen supplements.

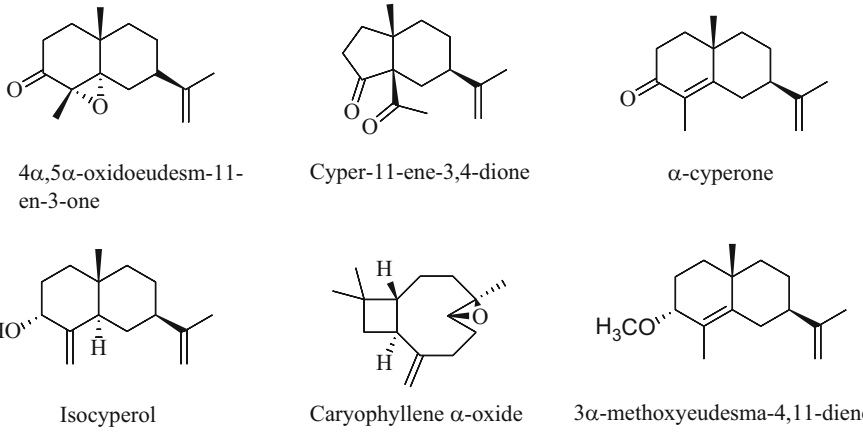


Fig. 20 Sesquiterpenes described as having estrogenic activity

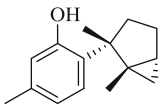


Fig. 21 Structure of the bacteriostatic compound in *L. okamurai*

14.9.3 Algae

There is a patent that concerns hair treatment compositions and methods of manufacturing the compositions for cosmetic use to prevent and reduce alopecia based on brown algae juice (Shim 2001). In this invention, the brown algae juice is mixed with juices of artemisia, pine needle, and other additives in order to obtain different compositions. A typical amount of brown algae juice is in the range of 30–60% of the total weight of the composition. It is hypothesized that the contents of I and Ca in the juice would have an effect on hormone secretion of the thyroid gland related to hair growth. However, none of the secondary metabolites of these algae and their possible effects are mentioned in this invention.

Another patent is related to the application of an algae sesquiterpenoid compound (Fig. 21) to prepare a bacteriostatic agent. This compound is extracted from *L. okamurai*, and it has strong inhibitory effect on *Streptococcus agalactiae* and *Vibrio anguillarum* (Jia et al. 2015).

14.9.4 Mushrooms

14.9.4.1 *Antrodia camphorata*

Antrocin, the main drimane-type sesquiterpenoid from *A. camphorate*, has shown promising anticancer results. A recent patent described medication containing antrocin to inhibit cancer cell growth as well as different bioassays performed to test its activity (Tzeng et al. 2012). It was demonstrated that treatment of breast cancer cells with antrocin would produce a selective inhibition of the cancerous cell proliferation.

14.10 Perspectives

Sesquiterpene lactones have attracted considerable interest since ancient times to treat several illnesses like inflammation, gastric disorders, or infections, and several of the associated metabolites are now in clinical trials, e.g., parthenolide or artemisinin. New efforts should be focused on the search for promising SL-derived drugs with improved physicochemical properties and bioavailability.

Although many sesquiterpenes are bioactive constituents of vegetables that have interesting health-promoting functions, more toxicological studies are required to evaluate accurately the toxicological effects of high quantities of these compounds in humans long-term. Nevertheless, the bitter tastes of these compounds might help to ascertain the appropriate amount of food containing sesquiterpene lactones that should be eaten on a daily basis.

Algae are eaten mainly in Eastern countries where they are integrated in the traditional gastronomy, but over time the consumption of algae is increasing in Western countries. Thanks to their nutritious contents, their use as a food in Europe and America has increased over the last few decades (Domínguez 2013). Given that many studies have highlighted the richness in beneficial secondary metabolites that these plants have, it is expected that their consumption will increase further over time.

14.11 Cross-References

- ▶ Anthocyanins in Food
- ▶ Antioxidants in Diets and Food
- ▶ Biflavonoids and Oligomeric Flavonoids from Food
- ▶ Chalcones in Diets
- ▶ Chlorogenic, Caffeic, and Ferulic Acids and Their Derivatives in Foods
- ▶ *Citrus* Flavanones
- ▶ Curcumin in Food
- ▶ Dietary Ellagitannins
- ▶ Dietary Fibers
- ▶ Dietary Flavonols and O-Glycosides
- ▶ Gallotannins in Food
- ▶ Gingerols and Shogaols from Food
- ▶ Lignans in Diets
- ▶ Polysaccharides in Food
- ▶ Prenylated Flavonoids in Food
- ▶ Saponins in Food
- ▶ Sesquiterpenes in Cereals and Spices
- ▶ Soy Isoflavones
- ▶ Tea Catechins
- ▶ Theaflavins, Thearubigins, and Theasinensins

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Sesquiterpenes in Cereals and Spices

15

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Abstract

This chapter provides an in-depth overview of the sesquiterpenes found in cereals and spices. These aliments provide an important source of nutrients for our health, and they are consumed daily. Thus, a knowledge of the constituents is vital to understand the origin of their benefits for human health as well as the possible side effects that can arise upon ingestion. Metabolite routes are detailed, being also provided a deep understanding of their numerous structures and bioactivities, to be used as food supplement or active ingredient in drugs. Among bioactivities found in the literature for these natural compounds, antioxidant, anti-inflammatory, or anticancer is remarkable.

Furthermore, we present a broad study of sesquiterpenes in cereals. We focus on trichothecenes, a family of mycotoxins, which represent a big concern worldwide. A study of their biotransformations in humans and animals, and their toxicity for the organism, is revised. In case of spices and miscellaneous food, a classification of their sesquiterpenes constituents present in the consumable parts was analyzed, showing the relevance of the structure variability in the activity.

Keywords

Dietary sesquiterpenes · Trichothecenes · Sesquiterpenes · Nutritive sesquiterpenes · Spices · Cereals · β-Caryophyllene · Sesquiterpene metabolism · Medicinal sesquiterpenes · Functional food

Abbreviations

15-MAS	15-Monoacetoxyscirpenol
3-ADON	3-Acetyldeoxynivalenol
4-DMAP	4-Dimethylaminopyridine
ADME	Absorption, distribution, metabolism, and excretion
ASNA	Adrenal sympathetic nerve activity
ATA	Alimentary toxic aleukemia
CCC	Countercurrent chromatography
COX	Cyclooxygenase
DAS	Diacetoxyscirpenol
DE 15-MAS	De-epoxy 15-monoacetoxyscirpenol
DE HT-2 toxin	De-epoxy HT-2 toxin
DE SCP	De-epoxy scirpentriol
DE T-2 tetraol	De-epoxy T-2 tetraol
DE T-2 triol	De-epoxy T-2 triol

DOM-1	De-epoxy deoxynivalenol
DOM-1GLU	Glucuronide conjugate derivative of DOM-1
DON	Deoxynivalenol
DONGLU	Glucuronide conjugate derivative of DON
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
ECD	Electron capture detector
FID	Flame ionization detector
GC	Gas chromatography
HPLC	High-performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
IgA	Immunoglobulins A
IgG	Immunoglobulins G
IgM	Immunoglobulins M
isoDON	Isodeoxynivalenol
LD_{50}	Median lethal dose
MCPBA	3-Chloroperbenzoic acid
MTS	Methylthiazole tetrazolium
NADPH	Hydronicotinamide-adenine dinucleotide phosphate
NEO	Neosolaniol
NICI	Negative ion chemical ionization
NIV	Nivalenol
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
PCI	Positive chemical ionization
PGE_2	Prostaglandin-E_2
RI	Retention indices
ROS	Reactive oxygen species
SCF	Scientific Committee on Food
SCP	Scirpentriol
SFC	Supercritical fluid chromatography
SFC/MS	Supercritical fluid chromatography-tandem mass spectroscopy
t-TDI	Temporary daily intake
TDI	Tolerable daily intake
TMS	Trimethylsilyl
TxB_2	Thromboxane B2
USSR	Union of Soviet Socialist Republics
UV	Ultraviolet

15.1 Introduction

This chapter provides an in-depth overview of the sesquiterpenes found in cereals and spices. These aliments provide an important source of nutrients for our health, and they are consumed daily. Thus, a knowledge of the constituents is vital to

understand the origin of their benefits for human health as well as the possible side effects that can arise upon ingestion.

Cereals represent one of the most common solid foods (Lawrie 1998); they play an important role in our diet and are an important source of nutrients, particularly for infants. However, there are numerous organisms that can damage or infect cereals both in the field or during storage and this causes many crops to be partially or entirely lost and presents a significant threat to our health. Among these threats it is worth highlighting trichothecenes. These are a family of mycotoxin sesquiterpenoids produced by several fungi from the genera *Fusarium*, *Stachybotrys*, *Myrothecium*, *Cephalosporium*, *Verticimonosporium*, *Trichoderma*, and *Trichothecium* during growth in host plants, food, and other environments (D'Mello et al. 1999; Grove 2007; Rocha et al. 2005). It has been demonstrated that industrial processing of cereal crops prior to their commercialization may decrease the level of these mycotoxins. However, such treatment does not completely eliminate these species (Kushiro 2008; Scudamore et al. 2009), and this results in humans being constantly exposed to these mycotoxins.

A total of 148 trichothecene structures have been isolated from natural sources, as stated by Grove (1986), but this number is increasing steadily. Some of the most important compounds of this family are deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), and T-2 and HT-2 toxins. Deoxynivalenol is most frequently detected in small cereal grains, and it is therefore becoming one of the biggest concerns worldwide due to its presence in many countries around the world (Placinta et al. 1999). The European Commission (Regulation 2007) established a regulation for maximum deoxynivalenol levels in different foodstuffs, and the Scientific Committee on Food (SCF) (European Commission 2005) established a Tolerable Daily Intake (TDI) for different mycotoxins produced by *Fusarium* species. Although deoxynivalenol is the most frequently found toxin, it is actually one of the least toxic compounds of this group (Rotter et al. 1996). Different studies on the effects of trichothecenes on animals have shown that other mycotoxins, for example, diacetoxyscirpenol and T-2 toxin, are generally more toxic than deoxynivalenol (Rocha et al. 2005; Thompson and Wannemacher 1986) and these are therefore a threat to humans and animals.

Concerning other foodstuffs, a wide range of plants are used to season different dishes in order to add or promote certain flavors and aromas. The use of spices and condiments in food has been known since ancient times, along with their use in medicinal applications. The development of chemical, physical, and biological technologies has demonstrated many properties of these natural additives, and it is possible to relate some benefits with specific compounds. In some cases, these natural substances are employed in food to provide additional qualities like color (e.g., saffron to give yellowish tones) or as preservatives (e.g., lemon juice to delay microorganism growth).

Most of the spices commonly used in cooking contain natural sesquiterpenes that we ingest and from which we obtain certain benefits. Examples include mint (*Mentha* spp.), pepper (*Piper nigrum*), cinnamon (*Cinnamomum verum*), basil (*Ocimum basilicum*), turmeric (*Curcuma longa*), bay laurel (*Laurus nobilis*), and salvia

(*Salvia officinalis*). The East Indian perennial herb *Curcuma amada* Roxb. is used in culinary applications to provide a raw mango flavor, for which it is commonly known as "mango ginger" (Sarikurkcu et al. 2018). Many medicinal and biological properties have been described for the rhizomes of this species in Indian folk medicine. For example, it is a remarkable appetizer, diuretic, antioxidant, anti-inflammatory, and cure for skin diseases, among other uses, and so it may be noteworthy for pharmacological applications (Policegoudra et al. 2011).

Numerous other examples of condiments used in food that are rich in sesquiterpenes can be found in nature: artemisia (*Artemisia umbelliformis*), mirto (*Myrtus communis*), satureja (*Satureja avromanica*), lemon (*Citrus limon*) and orange peel (*Citrus sinensis)*, mitsuba (*Cryptotaenia japonica* Hassk), and hatsudake (*Lactarius hatsudake*).

Mitsuba is a vegetable that is used as a topping in typical dishes of Eastern Asia, like the Japanese miso soup. This vegetable is characterized by its strong odor and is sometimes related with increased appetite. Sesquiterpenoids are the main components of mitsuba oil (Okuno et al. 2017).

Hatsudake is an edible and somewhat bitter mushroom that is also distributed in eastern areas of Asia like Japan, Korea, and China. This mushroom is employed as a base in Japanese traditional food, and it provides a green color and sweet and spicy flavors (Miyazawa et al. 2010).

15.2 Bioactive Constituents

15.2.1 Sesquiterpenes in Cereals

Trichothecenes are secondary metabolites produced by fungi. The different structures of trichothecenes are derived from a trichothecane (Grove 1986) skeleton (Fig. 1). The core structure of trichothecenes is formed by a fusion of a cyclohexene, tetrahydropyran and cyclopentyl rings, with a double bond at positions C-9 and C-10 and an epoxide group at positions C-12 and C-13, the latter of which is believed to be the cause of the toxicity (Zhou et al. 2008), are the base arrangement of trichothecenes. In this structure there are five positions, denoted as R1 to R5, that can bear different functional groups – most commonly acetyl and hydroxyl groups. Depending on the nature of these substituents trichothecenes are classified into four main groups (A, B, C, and D) (Foroud and Eudes 2009).

Type A trichothecenes stand out from the others due to the absence of a ketone group at C-8. The best known type A trichothecenes produced by the *Fusarium*

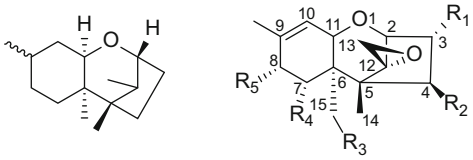
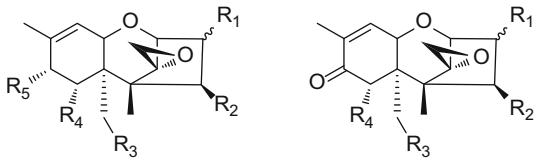


Fig. 1 Trichothacene (left) and base structure of trichothecenes (right)



Type A trichothecenes Type B trichothecenes

Compound	Skeleton	R1	R2	R3	R4	R5
T-2 toxin	Type A	α-OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 toxin	Type A	α-OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
Diacetoxyscirpenol	Type A	α-OH	OAc	OAc	H	H
Deoxynivalenol	Type B	α-OH	H	OH	OH	-
3-isomer-deoxynivalenol	Type B	β-OH	H	OH	OH	-
Nivalenol	Type B	α-OH	OH	OH	OH	-

Fig. 2 Structures of the best known trichothecenes

species are T-2 and HT-2 toxin, which have an isovalerate group at C-8. Type B trichothecenes have a ketone group in C-8, and this group of compounds includes deoxynivalenol and nivalenol. Type C trichothecenes are characterized by the presence of a second epoxy group at C-7/C-8 or C-9/C-10, and type D trichothecenes possess an ester-linked macrocycle between C-4 and C-16. Trichothecenes from groups A and B are the most widely studied because they are detected in naturally contaminated foods (Côté et al. 1984). The structures of the best known trichothecenes are provided in Fig. 2.

Trichothecenes are nonvolatile and are very resistant to thermal and light degradation, which makes it difficult to remove these compounds during the industrial processes of food elaboration. However, there are several different methods that can be employed to modify their structures to produce less toxic derivatives.

Decontamination of Trichothecenes

Trichothecenes have proven to have high heat resistance (Pronyk et al. 2006) and to resist neutral and acidic pH conditions (Rocha et al. 2005). However, there are several approaches to decontaminate these compounds. The most effective way seems to involve the removal of the epoxy group at C-12/C-13 and/or the double bond at C-9/C-10, both of which are believed to play an important role in the toxicity of these compounds (Fig. 1).

15.2.1.1 Chemical Methods for the Decontamination of Trichothecenes

Oxidation Under Alkaline Conditions

The treatment of deoxynivalenol and nivalenol under alkaline conditions will lead to rupture of the 12,13-epoxy group or the elimination of the C-15 atom as formaldehyde through a retroaldol rearrangement (Bretz et al. 2006).

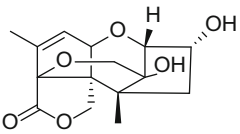


Fig. 3 Structure of Grove's lactone of DON

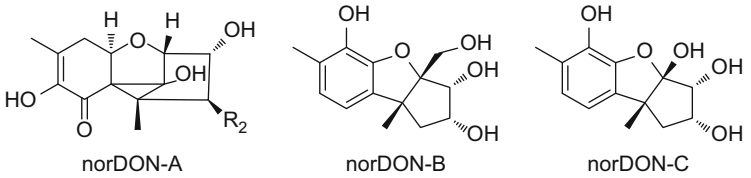


Fig. 4 Structures of the products obtained by treatment of DON under alkaline conditions at 75 °C

Alkaline hydroxylation of deoxynivalenol with 0.1 M methanolic NaOH at room temperature produces a rearrangement in the cyclohexene ring that would lead to the opening of the epoxide group to give a lactone (Fig. 3) (Grove 1985).

Grove et al. carried out the hydroxylation of deoxynivalenol at room temperature, but when the same reaction was carried out by Young et al. at 75 °C, the opening of the epoxy group produced different degradation products (Young 1986). Four new derivatives were detected, namely, norDON-A, norDON-B, norDON-C (Fig. 4), and traces of a compound believed to be the lactone described by Grove.

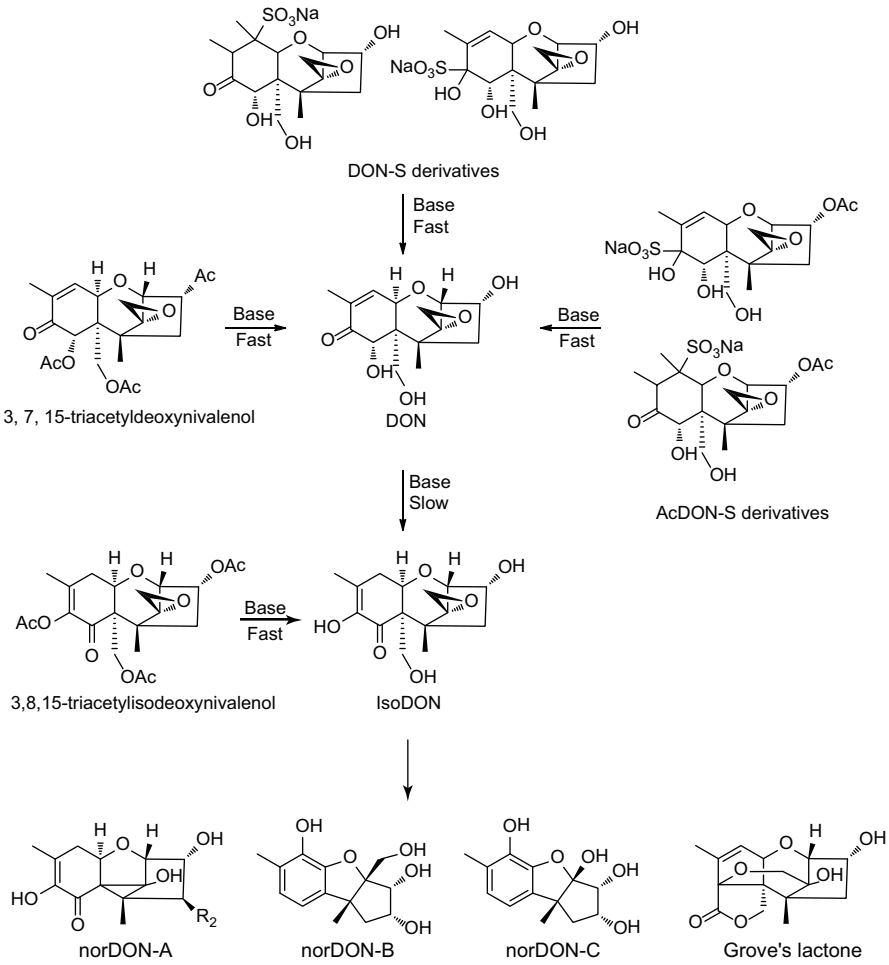
As shown in Scheme 1, alkaline hydroxylation under the same conditions of acetyl and sulfonate derivatives of deoxynivalenol led to rapid hydroxylation to form deoxynivalenol, which is subsequently converted to isodeoxynivalenol (isoDON) and then quickly degraded to the three norDON derivates shown. 3,7,15-Triacetyldeoxynivalenol displayed different behavior and was hydroxylated directly to isodeoxynivalenol (Young 1986). Similar results were obtained when nivalenol was treated under the same conditions, with the equivalent compounds formed for nivalenol of norDON-A, norDON-B, norDON-C, and Grove's lactone.

A process involving heating with *N*-α-acetyl-L-lysine methyl ester would also remove the epoxide group of deoxynivalenol to produce different derivatives (Bretz et al. 2006). In this work five known degradation compounds of deoxynivalenol were observed: isodeoxynivalenol, norDON-A, norDON-B, norDON-C, and Grove's lactone. Four new degradation compounds were observed: 9-hydroxymethyl DON lactone, norDON-D, norDON-E, and norDON-F (Fig. 5).

Oxidation of deoxynivalenol with NaOCl would not open the epoxide in positions C-12 and C-13 but would lead to the formation of another epoxide group at positions C-9 and C-10. An unusual 9α,10α-configuration and a hemiketal in positions C-8 and C-15 were observed (Fig. 6) (Burrows and Szafraniec 1987).

Ozonolysis

Ozonolysis of the trichothecene skeleton takes place at the C-9 and C-10 double bond. The degradation results in the opening of the cyclohexene ring by the mechanism presented in Scheme 2 (Young et al. 2006).



Scheme 1 Alkaline hydrolysis of acetyl and sulfonate derivatives of deoxynivalenol

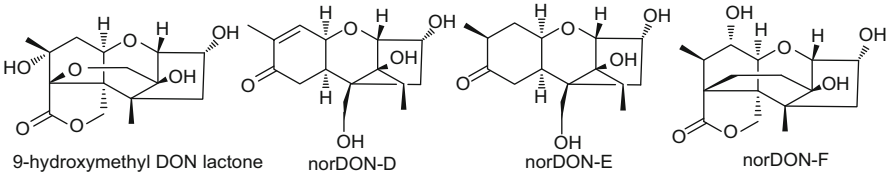


Fig. 5 Structures of 9-hydroxymethyl DON lactone, norDON-D, norDON-E, and norDON-F

Reduction

Reduction of trichothecenes with sodium bisulfite ($NaHSO_3$) and sodium metabisulfite ($Na_2S_2O_5$) produces derivatives with lower toxicity than the original

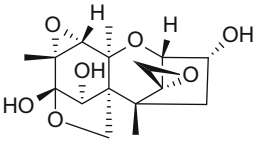
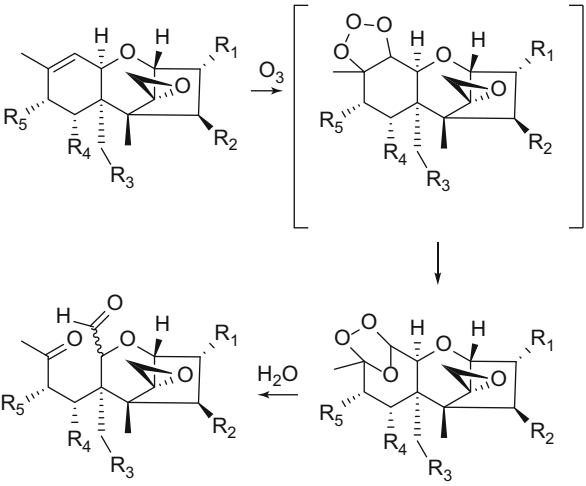


Fig. 6 Structure of the product obtained from the oxidation of DON with NaOCl



Scheme 2 Mechanism proposed for the opening of the cyclohexene ring during ozonolysis of trichothecenes

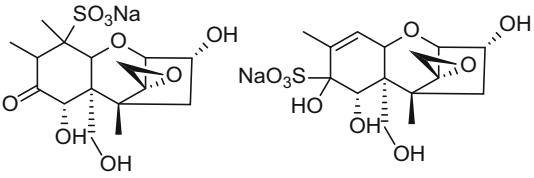
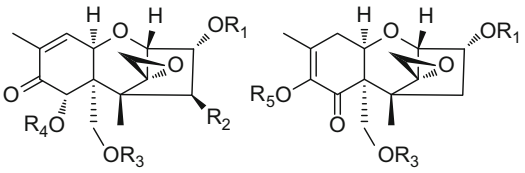


Fig. 7 Structures of deoxynivalenol sulfonate derivatives

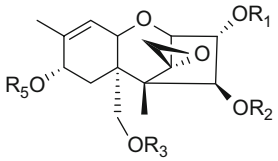
trichothecenes. The reactivity of $Na_2S_2O_5$ with different trichothecenes has been studied (Young 1986), and this reaction produces different sulfonate derivatives. Bisulfites usually react with α,β-unsaturated enones in the keto- or β-positions. In the case of deoxynivalenol, the reduction gives sulfonate derivatives in positions 8 and 10 (Fig. 7).

The reaction seems to be influenced by the substituents on adjacent groups, and it is particularly fast in the cases of deoxynivalenol and nivalenol, which bear a hydroxyl group in positions C-7 and C-15, respectively. Nevertheless, the reaction is slower for 3,15-diacetyldeoxynivalenol, which has an acetyl group in position C-15. Isodeoxynivalenol, with an 8-enol-7-one system, reacted very slowly, and 3,8,15-triacetylisodeoxynivalenol did not react at all. These results seem to indicate that steric factors around the ketone group play an important role in the mechanism of the reaction. Similar results were obtained when the same reaction conditions were applied to nivalenol. The chemical environments of the α,β-unsaturated enones of different trichothecenes are shown in Fig. 8.



Compound	Skeleton	R1	R2	R3	R4	R5	Rate
Deoxynivalenol	A	H	H	H	H	-	Fast
Nivalenol	A	H	OH	H	H	-	Fast
3,15-diacetyldeoxynivalenol	A	Ac	H	Ac	H	-	Slow
Isodeoxynivalenol	B	H	-	H	-	H	Very slow
3,8,15-triacetylisodeoxynivalenol	B	Ac	-	Ac	-	Ac	No reaction

Fig. 8 Structures of different deoxynivalenol and nivalenol derivatives. Their substituents play an important role in the reduction with sulfites



Compound	R1	R2	R3	R4	R5
T-2 toxin	H	Ac	Ac	H	$COCH_2CH(CH_3)_2$
HT-2 toxin	H	H	Ac	H	$COCH_2CH(CH_3)_2$
3'-OH T-2 toxin	H	Ac	Ac	H	$COCH_2COH(CH_3)_2$
3'-OH HT-2 toxin	H	H	Ac	H	$COCH_2COH(CH_3)_2$

Fig. 9 Structures of T-2 and HT-2 toxin and its 3′-OH derivatives

15.2.1.2 Biological Methods for the Decontamination of Trichothecenes

There are reports concerning biotransformations of trichothecenes by biological systems (Ueno et al. 1983; Westlake et al. 1987), and this offers an interesting and more environmentally friendly alternative for the detoxification of these compounds.

Oxidation

Animals catalyze the hydroxylation of type A trichothecenes produced by the addition of a hydroxyl group to position C-3′ of the substituent in the C-8 position (Corley et al. 1986). Thus, 3′-hydroxy T-2 toxin and 3′-hydroxy HT-2 toxin (Fig. 9) have been identified in different animals such as swine, cow, or rat. This hydroxylation reaction takes place in microsomes. Dihydronicotinamide adenine dinucleotide phosphate (NADPH) is required, and the reaction is catalyzed by cytochrome P450. The presence of a chemical called phenobarbital, which is produced in the liver, enhances the hydroxylation procedure (He et al. 2010).

Deoxynivalenol can also be oxidized by the soil bacterium strain E3-39 (*Agrobacterium-Rhizobium* spp.) to obtain a derivative known as 3-keto-DON

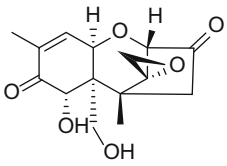


Fig. 10 Structure of 3-keto-DON

(Fig. 10) (Shima et al. 1997). This compound has shown immunosuppressive activity that is ten times lower than that of deoxynivalenol.

Reduction

As mentioned previously, the epoxide group at position C-12 and C-13 is responsible for the toxicity of trichothecenes. It has been shown that the de-epoxy forms of trichothecenes are less toxic than their respective trichothecenes; for example, de-epoxy deoxynivalenol (DOM-1) and de-epoxy nivalenol showed 54 and 55 times lower cytotoxic activity than their epoxy forms (Sundstøl Eriksen et al. 2004).

Many animals can metabolize trichothecenes and remove the epoxide group by reduction to a double bond. However, substitution in position C-4 has been shown to hinder this reaction. This is the case for T-2 toxin, diacetoxyscirpenol, and neosolaniol (NEO), which have an acetyl group in the C-4 position. Hydrolysis of this acetyl group is faster than the de-epoxidation, and this makes it difficult to detect the direct de-epoxy form. Cow rumen microorganisms metabolize deacetoxyscirpenol to give four products: 15-monoacetoxyscirpenol (15-MAS), scirpentriol (SCP), and their de-epoxy forms (DE 15-MAS and DE SCP) (Swanson et al. 1987a). On the other hand, bovine rumen microorganisms have metabolized T-2 toxin into de-epoxy HT-2 toxin (DE HT-2 toxin), de-epoxy T-2 triol (DE T-2 triol), and de-epoxy T-2 tetraol (DE T-2 tetraol), which were also less toxic than their original trichothecenes (Swanson et al. 1987b). The lethal doses to brine shrimp of T-2 toxin and deacetoxyscirpenol, as well as their epoxy and de-epoxy derivatives (Fig. 11) obtained from bovine rumen metabolization, are provided in Table 1 (Swanson et al. 1987b). There are also various nonruminant animals, such as rats, that can also reduce deoxynivalenol into DOM-1 (Yoshizawa et al. 1983).

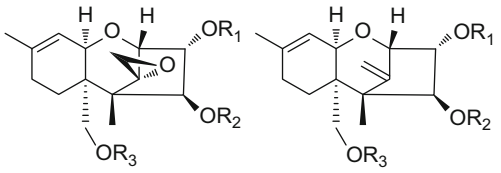
Hydrolysis

Acetyl groups are common functional groups in the structures of trichothecenes. Their number and position play an important role in the activity of the compounds (Betina 1989). Hydrolysis of these acetyl groups is a common reaction in biochemical pathways involving animals and microorganisms (rumen microflora).

Deoxynivalenol, diacetoxyscirpenol, and T-2 toxin were treated with sheep rumen fluid, and this led to deacetylation of DAS and T-2 Toxin, but DON remained unaltered. As a result, DAS was transformed into 15-monoacetoxyscirpenol and T-2 toxin into HT-2 toxin. Sheep rumen fluid did not have any effect on DON (Kiessling et al. 1984).

Another example of hydrolysis can be found in *Curtobacterium* spp. Strain 114-2. This is a soil bacterium that can hydrolyze T-2 toxin into HT-2 toxin (He

Fig. 11 Structures of deoxyscirpenol derivatives obtained by the metabolization of DAS by cow rumen microorganisms



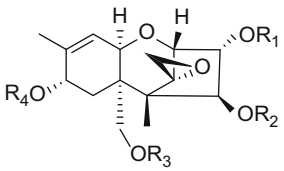
Skeleton A Skeleton B

Compound	Skeleton	R1	R2	R3
DAS	A	H	Ac	Ac
15-MAS	A	H	H	Ac
SCP	A	H	H	H
DE 15-MAS	B	H	H	Ac
DE SCP	B	H	H	H

Table 1 Removal of the epoxide group and increased of median lethal dose (LD_{50}) values (Swanson et al. 1987b)

Compound	LD_{50} (ng/mL)	Compound	LD_{50} (ng/mL)
DAS	178	**T-2 toxin**	111
15-MAS	128	**HT-2 toxin**	258
SCP	6000	**T-2 triol**	1377
DE 15-MAS	≫6000	**T-2 tetraol**	964
DE SCP	≫6000	**DE HT-2 toxin**	>6000
–	–	**DE T-2 triol**	>5000
–	–	**DE T-2 tetraol**	≫6000

Fig. 12 Structures of T-2 toxin derivatives



Compound	R1	R2	R3	R4
T-2 toxin	H	Ac	Ac	$OCOCH_2CH(CH_3)_2$
HT-2 toxin	H	H	Ac	$OCOCH_2CH(CH_3)_2$
T-2 triol	H	H	H	$OCOCH_2CH(CH_3)_2$
T-2 tetraol	H	H	H	H

et al. 2010), which is further hydrolyzed to T-2 triol. T-2 triol can subsequently be deacetylated to T-2 tetraol by the rumen bacterium strain BBSH 797 (Fig. 12) (Fuchs 2002).

Different strains of rumen bacteria like *Butyrivibrio fibrisolvens* (cow, deer, and sheep), *Selenomonas ruminantium*, and *Anaerovibrio lipolytica*, which can be isolated from ruminants (cow and sheep), have been tested on T-2 toxin. The results

show that these bacteria can metabolize T-2 toxin into HT-2 toxin, T-2 triol, and neosolaniol with different percentages of transformation (Westlake et al. 1987).

Hydration

In the hydration reaction, a molecule of water is consumed to add a hydroxyl group and a proton to an unsaturated substrate, e.g., an alkene. A strain of *Aspergillus* spp. (*NJA-1*) is able to transform deoxynivalenol into a product that has a mass 18.1 Da higher than DON (Chenghua et al. 2008). The authors suggested that the new molecule would be the result of a hydration reaction, but the chemical structure was not determined.

Conjugation by Glycosylation

In a glycosylation reaction, a glycosyl donor interacts with a glycosyl acceptor to produce a glycoside. Trichothecenes can be conjugated by glycosylation to form glucuronides and glucosides.

Trichothecene glucuronides are molecules formed by the union of a glucuronic acid and a trichothecene through a glycosidic bond, generally with a hydroxyl group located at C-3 of the trichothecene. The glucuronidation derivative seems to be produced by UDP-glucuronosyltransferases. Furthermore, cows are able to metabolize DON and DOM-1 into their glucuronide conjugates (DONGLU and DOM-1GLU) (Côté et al. 1986). On the other hand, swine have proven to be able to metabolize T-2 toxin to give glucuronide conjugates of HT-2 toxin, 3′-OH T-2 toxin, 3′-OH HT-2 toxin, and T-2 toxin among other metabolized products (Corley et al. 1985). Rats are also able to metabolize T-2 toxin and DAS to obtain the glucuronic HT-2 toxin, among other glucuronic derivatives of T-2 toxin. It is believed that a previous deacetylation occurs before the conjugation with glucuronic acid (Gareis et al. 1986), and the glucuronic derivatives of 15-MAS and scirpentriol were obtained from DAS.

Trichothecene glucosides are molecules that result from the union of a trichothecene and the anomeric carbon of a glucose through a glycosidic bond. A UDP-glycotransferase isolated from *Arabidopsis thaliana* catalyzes the transference of glucose from UDP-glucose to the hydroxyl group at C-3 of DON to produce DON-3-glucoside (Poppenberger et al. 2003).

Trichothecene glucuronide and glucoside derivatives of trichothecenes have lower toxicity than their non-derivated counterparts. A methylthiazole tetrazolium (MTS) cell viability assay to study the cytotoxic activity on a human erythroleukemia cell line K562 of DON and DONGLU showed that DON produced 50% inhibition of cell numbers at a concentration of 1.31 μM. In contrast, DONGLU did not show any relevant cytotoxicity up to 270 μM (Wu et al. 2007). The phytotoxic activities of DON and DON-3-glycoside to inhibit protein synthesis of wheat ribosomes was evaluated, and it was shown that DON-3-glycoside was less toxic than DON (Poppenberger et al. 2003).

Combination of Bioprocesses

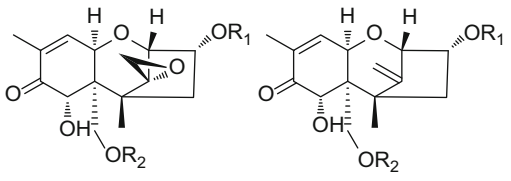
There are numerous possible biotransformations of trichothecenes, and it is difficult to envisage that when an animal eats food, it will follow only one route of metabolization. On the contrary, the food undergoes different metabolization processes to give a variety of metabolites.

Côté (Côté et al. 1986) analyzed the ability of cow rumen fluid to reduce the epoxy group of DON to obtain DOM-1. However, it was found that cow rumen fluid possesses microorganisms that are capable of transforming 3-acetyldeoxynivalenol (3-ADON) into DOM-1, where DON is an intermediate product (Fig. 13) (King et al. 1984). Thus, deacetylation and de-epoxidation reactions take place in the process.

Eubacterium strain BBSH 797 is the most widely studied bacterium isolated from bovine rumen fluid, and it is responsible for the transformation of DON into DOM-1 (Schatzmayr et al. 2006), which is less toxic than DON. This strain can also transform type A trichothecenes through de-epoxidation and/or deacetylation (Fuchs 2002). T-2 toxin is hydrolyzed to HT-2 toxin in the presence of this strain. HT-2 toxin, T-2 tetraol, and scirpentriol experience a de-epoxidation to give their de-epoxy forms. T-2 triol is metabolized through two different paths: the first one leads to de-epoxy T-2 triol through a de-epoxidation and in the second path T-2 triol is hydrolyzed to T-2 tetraol. Finally, the de-epoxide form is reached.

Incubation of trichothecenes in the presence of fecal and intestinal microflora of different animals was carried out in anaerobic conditions, and this resulted in a diverse range of biotransformations (Swanson 1988). The results showed that rats, swine, and cattle completely transformed DAS into de-epoxy 15-monoacetoxyscirpenol and de-epoxyscirpentriol through a deacetylation and de-epoxidation pathway. Chicken, horses, and dogs managed to produce the deacetylation products (15-MAS and SCP) from DAS but failed to reduce the epoxide group. Rats showed a high metabolization activity to transform T-2 toxin into de-epoxy HT-2 toxin and T-2 triol, in addition to transforming T-2 tetraol and SCP into their de-epoxy forms.

It is important to note that the metabolization of trichothecenes may change markedly between animal varieties of the same species. For example, in contrast to the results obtained by Swanson et al., which were discussed in the last paragraph,



Skeleton A Skeleton B

Fig. 13 Structures of DON, 3-ADON, and DOM-1

Compound	Skeleton	R1	R2
Deoxynivalenol	A	H	H
3-acetyldeoxynivalenol	A	Ac	H
De-epoxydeoxynivalenol	B	H	H

chickens showed an effective de-epoxidation of DON into DOM-1 (He et al. 1992). In another case, swine managed to transform DON into DOM-1 (Kollarczik et al. 1994), whereas metabolization was not observed (He et al. 1992).

The most important metabolization routes found for sesquiterpenes in cereals are summarized in Table 2.

15.2.2 Sesquiterpenes in Spices and Herbs

Among the bioactive compounds that have been identified in food (e.g., glucosides, fatty acids, monoterpenes, or diterpenes), sesquiterpenes are remarkable due to their

Table 2 Metabolic transformations of trichothecenes

Substrate	Bioorganism	Product	Transformation	Reference
T-2 toxin	Cow, rat, and swine	3′-OH T-2 toxin	Hydroxylation	Corley et al. (1986)
	Bovine rumen	DE HT-2 toxin, DE T-2 triol, and DE T-2 tetraol	Hydroxylation, reduction	Swanson et al. (1987b)
	Sheep	HT-2 toxin	Hydrolysis	Kiessling et al. (1984)
	Curtobacterium spp.	HT-2 toxin and T-2 triol	Hydrolysis	He et al. (2010)
	Butyrivibrio fibrisolvens (cow, sheep, deer), *Selenomonas ruminantium* and *Anaerovibrio lipolytica* (cow, sheep)	HT-2 toxin, T-2 triol, T-2 tetraol, and NEO	Hydrolysis	Westlake et al. (1987)
	Swine	Glucuronide derivative of T-2 toxin, HT-2 toxin, 3′-OH T-2 toxin, and 3′-OH HT-2 toxin	Hydrolysis, hydroxylation and conjugation by glycosylation	Corley et al. (1985)
	Rat	Glucuronide derivate of HT-2 toxin	Hydrolysis and conjugation by glycosylation	Gareis et al. (1986)
		DE HT-2, DE T-2 triol, and DE T-2 tetraol	Hydrolysis and reduction	Swanson (1988)
	Eubacterium strain BBSH 797	HT-2 toxin, DE T-2 triol, and DE T-2 tetraol	Hydrolysis and reduction	Fuchs (2002)
HT-2 toxin[a]	Cow, rat, and swine	3′-OH HT-2 toxin	Hydroxylation	Corley et al. (1986)

(continued)

Table 2 (continued)

Substrate	Bioorganism	Product	Transformation	Reference
DON	*Agrobacterium-Rhizobium* sp.	3-keto-DON	Oxidation	Shima et al. (1997)
	Rat	DOM-1	Reduction	Yoshizawa et al. (1983)
	Aspergillus sp.	Hydrated DON	Hydration	Chenghua et al. (2008)
	Cow	DONGLU	Conjugation by glycosylation	Côté et al. (1986)
		DOM-1GLU	Reduction and conjugation by glycosylation	Côté et al. (1986)
	Arabidopsis thaliana	DON-3-glycoside	Conjugation by glycosylation	Poppenberger et al. (2003)
	Eubacterium strain BBSH 797 (bovine rumen)	DOM-1	Reduction	Schatzmayr et al. (2006)
	Chicken	DOM-1	Reduction	He et al. (1992)
	Swine	DOM-1	Reduction	Kollarczik et al. (1994)
DAS	Cow	15-MAS, SCP, DE 15-MAS, and DE SCP	Hydrolysis and reduction	Swanson et al. (1987a)
	Sheep	15-MAS	Hydrolysis	Kiessling et al. (1984)
	Rat	Glucuronide derivate of 15-MAS and SCP	Hydrolysis and conjugation by glycosylation	Gareis et al. (1986)
		DE 15-MAS and DE SCP	Hydrolysis and reduction	Swanson (1988)
	Swine and cattle	DE 15-MAS and DE SCP	Hydrolysis and reduction	Swanson (1988)
	Chicken, horse, and dog	15-MAS and SCP	Hydrolysis	Swanson (1988)

[a]It is important to note that in many cases during metabolization, T-2 toxin transforms into HT-2 toxin through a hydrolysis reaction, and from this point HT-2 toxin follows different metabolization routes. For this reason, even though only one transformation has been listed for HT-2 toxin in the table, more information about its metabolization can be found in the T-2 toxin data in the table.

prominent presence, the role they execute in the plant (and in the environment), and the wide range of activities that they have shown in numerous studies.

When sesquiterpenes are classified according to their skeleton, more than 100 families have been established. The structural variability of these compounds covers a wide range, with differences in the number and size of rings, the presence of aromaticity and/or functional groups, multiplicity of bonds, or isomerism. This

variety of structures provides this family of compounds with a wide range of activities that can provide health benefits when they are consumed in food.

Sesquiterpenoids have been widely studied in herbs and spices over the years because of the profitable properties that these foods show. Among the sesquiterpenoids present, the caryophyllane and humulene skeletons are the most common. Nevertheless, the variety of herbs employed in different cultures means that, within the spices group, there are more than 40 relevant sesquiterpenoids.

Among the sesquiterpenes found in spices and herbs, it is worth highlighting elemanes, cardinanes, drimanes, daucanes, guaianes, eudesmanes, and germacrenes.

15.2.2.1 β-Caryophyllene and Derivatives

β-Caryophyllene is one of the most relevant compounds. This sesquiterpene is related with spiciness in food and this explains its common presence (Jirovetz et al. 2002). The structure of β-caryophyllene contains a cyclobutane ring (Fig. 14), which is very unusual in nature, and only a few compounds such as pentacycloanammoxic acid (Mascitti and Corey 2006; Sinninghe Damste 2002) have this skeleton. In the case of α-humulene, previously known as α-caryophyllene, the morphology is similar to β-caryophyllene, but the cyclobutane ring has been opened (Vedernikov and Roshchin 2011). Other examples of humulene structures include zerumbone, which is obtained from ginger plants, and a relevant group of aziridine derivatives with promising anticancer activity have been synthesized (Gopalan et al. 2017).

Within the zerumbone compounds (Fig. 15), a large number of oxidized derivatives have attracted attention, and many new structures have been defined recently. Triepoxyzerumbol is one such compound and it is synthesized from zerumbone using a lipase catalyst. Another example is (2*R*)-tetrahydrozerumbone, which is prepared in a facile procedure with 5% Pd/C and H_2 from zerumbone. Furthermore, (2*R*)-tetrahydrozerumbone has a powerful balmy fragrance. Finally, 6,7-monoepoxyzerumbone can be obtained by oxidation of monoepoxyzerumbol, which is obtained by epoxidation of zerumbone with MCPBA (3-chloroperbenzoic acid) in ethyl acetate (Kitayama et al. 2007, 2008, 2012).

14-Hydroxy-β-caryophyllene, (*3Z*)-caryophylla-3,8(13)diene-5-ol, and caryophylla-4(12),8(13)diene-5-ol (Fig. 15) are oxygenated β-caryophyllene derivatives present in hops, and these are differentiated by the presence of a hydroxymethyl moiety at the cyclobutane or cyclononane ring (Praet et al. 2016).

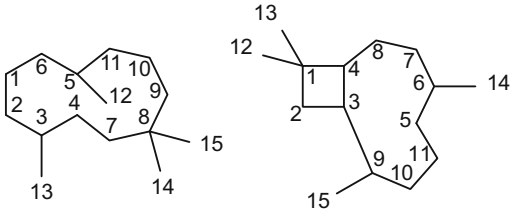


Fig. 14 Carbon skeletons of humulene (left) and caryophyllane (right) structures

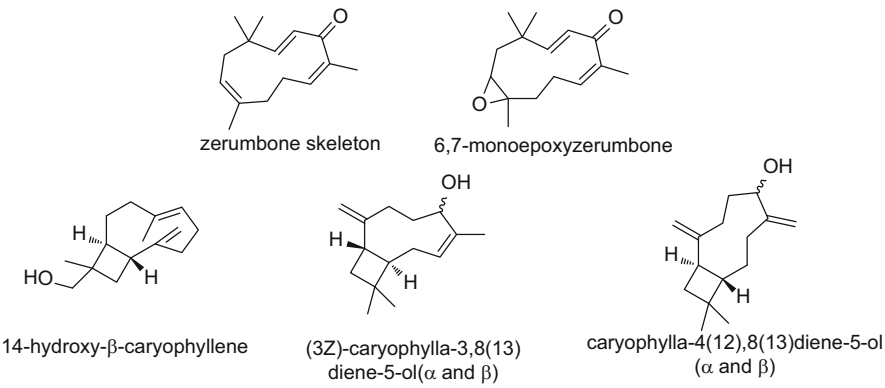


Fig. 15 Zerumbone skeleton (top left), 6,7-monoepoxyzerumbone (top right), and structures of some β-caryophyllene derivatives (bottom)

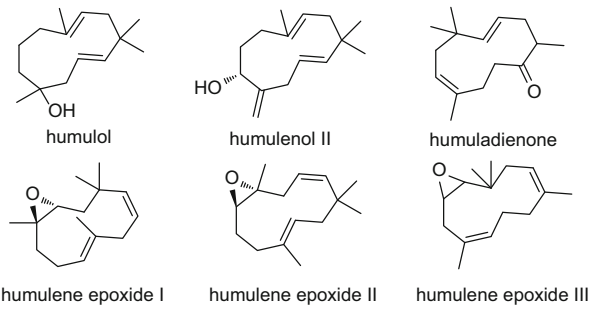


Fig. 16 Structures of humulene derivatives found in beer aroma (stereochemistry is not shown in cases where it is not known)

Hop essential oil also provides two important humulenes for beer aroma: humulenol II and humulene epoxide III. Besides, more humulene-skeleton products have been identified as minor components: humulol, humulene epoxides I and II, and humuladienone (Praet et al. 2016). The structures of these compounds are shown in Fig. 16.

15.2.2.2 Elemanes and Cadinanes

The elemane and cadinane families also cover a large number of spice sesquiterpenoids. The synthetic procedure for these compounds starts with a Cope sigmatropic rearrangement from 1(10),4-germacradienes (Barrero et al. 1999; Akatsuka et al. 1993), with the elemane configuration generally more stable than that of germacranes (Barquera-Lozada and Cuevas 2017). The principal skeleton of these compounds is a cyclohexene unit, like β-elemene and δ-elemene, in which a double bond has been displaced (Fig. 17).

Cadinanes are formed by two fused six-membered rings with an isopropyl group on the carbon next to the fusion point. Cubenol and calamenene are among the main cadinanes present in herbs, such as *Mentha* spp., and they have recently

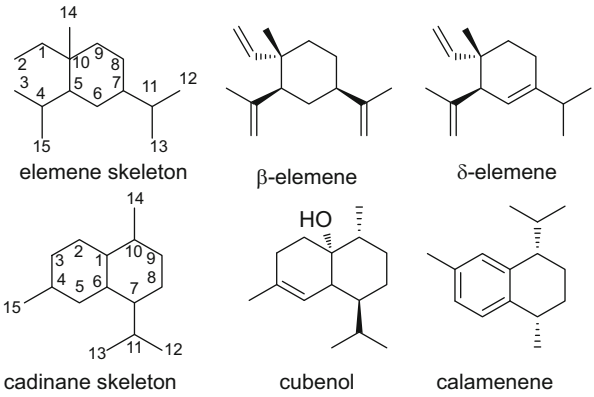


Fig. 17 Carbon skeleton of elemene structures (top left) and two important elemenes in spices (top right). Carbon skeleton of cadinane structures (bottom left) and relevant cadinanes in herbs (bottom right)

Table 3 Sesquiterpenes present in *Laurus nobilis* L. essential oil detected by GC-MS analysis

Sesquiterpene hydrocarbons	Isoledene α-Copaene β-Elemene β-Caryophyllene α-Guaiene α-Humulene Germacrene D β-Selinene α-Selinene α-Bulnesene 6,9-Guaiadiene *Allo*-aromadendrene Bicyclogermacrene γ-Cadinene *Trans*-cadina-1,4-diene
Oxygenated sesquiterpenes	Spathulenol Caryophyllene oxide Viridiflorol Ledol β-Eudesmol α-Eudesmol α-Cadinol

shown repellent activity against *Curculionidae* Coleoptera (Espinoza et al. 2016) and human benefits that will be discussed later. These cadinanes are also of chemical interest, and some derivatives of natural calamenene have been reported to be formed by an intramolecular Friedel–Crafts-type Michael addition (Sun et al. 2010).

β-Elemene can be found in bay leaf (Table 3), and γ-elemene is present in peppermint essential oil as the second major sesquiterpene after β-caryophyllene (Orav and Kann 2001).

15.2.2.3 Drimanes and Coloratanes

The drimane sesquiterpene skeleton also contains two fused six-membered rings, but it is characterized by a higher number of methyl substituents (Fig. 18). These usually have a highly oxidized structure, as in the case of *Warburgia ugandensis* and *W. stuhlmannii*, from which three dialdehyde drimanes have been isolated. Furthermore, the natural product drimenal, which has antifungal and antibacterial activities, has recently been synthesized (Li et al. 2018a). Muzigadial has also been isolated from *Warbugia* spp. together with polygodial, warbuganal, and ugandensidial, and it has a coloratane carbon sesquiterpene structure (Kubo and Ganjian 1981).

A new derivative of polygodial, 1-(*R*)-hydroxypolygodial, has recently been synthesized, and this has shown relevant results against vanilloid receptors in HEK cells. The different locations of hydroxyl groups in polygodial derivatives were tested, and only the 1-(*R*)-hydroxy derivative showed bioactivity (D'Acunto et al. 2010).

15.2.2.4 Guaianes, Daucanes, and Aromadendranes

Within the sesquiterpenoids, guaiane structures are broadly distributed as secondary metabolites in plants. Sesquiterpene lactones are a specific family of sesquiterpenoids that show wide biological activities in different respects and living organisms (Macías et al. 2007). The skeleton has a five-membered ring fused with a seven-membered ring that has an isopropyl group in the β-position to ring fusion. The presence of double bonds in the cycloheptane ring, along with their number and location, provides a wide variety of guaiane structures (Fig. 19). A good example is α-bulnesene and α-guaiene which have a double bond in different locations. These two molecules are present in black pepper (Nikolic et al. 2015) and also in sweet basil (Nurzyńska-Wierdak 2012). Rotundone is a sesquiterpene derived from the oxidation of α-guaiene, and it is differentiated by the presence of a carbonyl group in

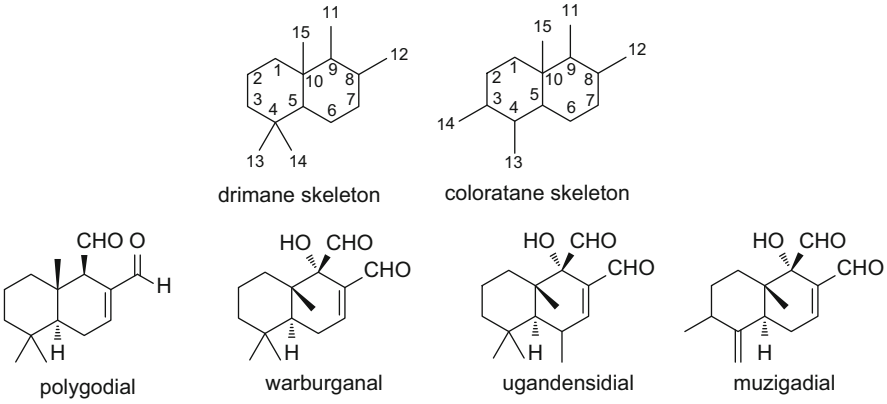


Fig. 18 Drimane skeleton (top left), coloratane sesquiterpene structure (top right), and four relevant drimanes and coloratanes found in ginger plants

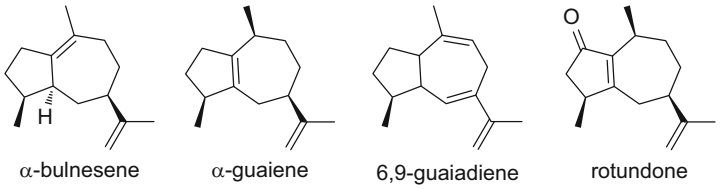


Fig. 19 Numerous structures arise from the variation of the number and location of double bonds in the cycloheptane ring

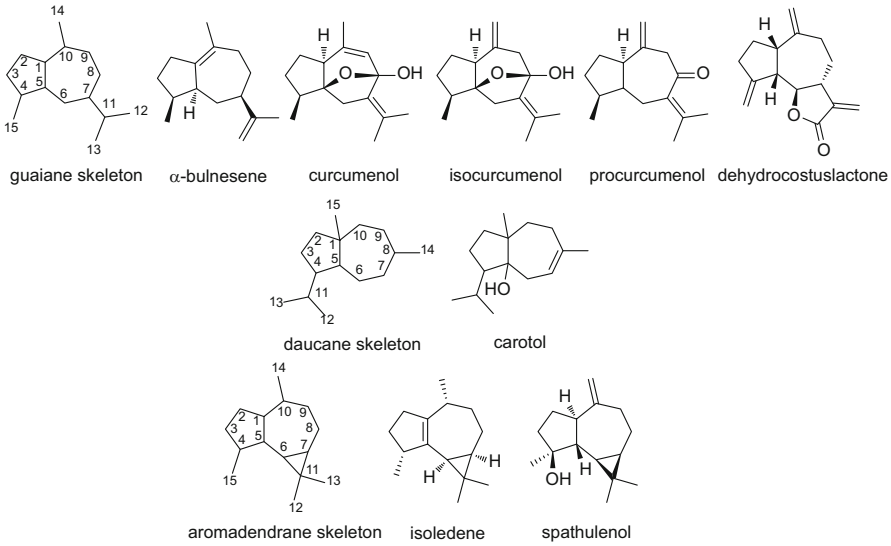


Fig. 20 Guaiane-type sesquiterpene structure (top left) and relevant guaianes isolated from herbs (top right). Daucane skeleton (middle left) and daucanes found in Chinese spice. Aromadendrane skeleton (bottom left) and relevant aromadendrenes isolated from herbs

the pentene ring. Rotundone contributes significantly to pepper aroma, and it also provides characteristic aromas in red wines made from Shiraz grapes. The odor detection threshold of this compound is one of the lowest of all natural products (Wood et al. 2008).

A relevant guaiane sesquiterpene synthesized by holy basil (*Ocimum sanctum*) is α-bulnesene (Suanarunsawat et al. 2009, 2010). Notwithstanding, the highest number of guaianes are isolated from turmeric plants (Zingiberaceae family), which contain curcumenol and related derivatives (Morikawa 2007) (Fig. 20). Daucane-type sesquiterpenoids are very similar to guaiane and differ only in a translocation of the isopropyl group in the five-membered ring and a rearrangement of the positions of the methyl groups. Carotol, isolated from *Alpinia guinanensis* (a food spice in China), is an example of a daucane found in food (Deng et al. 2016).

Aromadendranes have a similar carbon skeleton to guaiane-type compounds, but the most oxidized carbon of the isopropyl group is linked to the seven-membered ring to create a cyclopropane (Fig. 20). These three fused rings (cyclopropane, cycloheptane, and cyclopentane) shape aromadendrane sesquiterpenes. Isoledene is a major aromadendrane in *Mentha* spp. (Park et al. 2016), and spathulenol is the main constituent of the essential oil of *Satureja avromanica* (Iranian spice). Aromadendranes generate guaine sesquiterpenes by a ring opening of the cyclopropane, which leads to the formation of an isopropyl group. This can be easily observed when isoledene is treated with HSO_3F-SO_2FCl or $BF_3{\cdot}Et_2O$ to generate isoguaiene (Moreno-Dorado et al. 2003).

15.2.2.5 Eudesmanes, Secoeudesmanes, and Eremophilanes

Eudesmane-type sesquiterpenoids have a similar skeleton to cadinane, but the isopropyl group is found in the β-position with respect to ring fusion, and these compounds are characterized by a methyl group at C-10.

α-Eudesmol and β-eudesmol (Fig. 21) are two eudesmanolides with a hydroxyl group on the isopropyl group. Both molecules are present in bay leaf, as detailed in

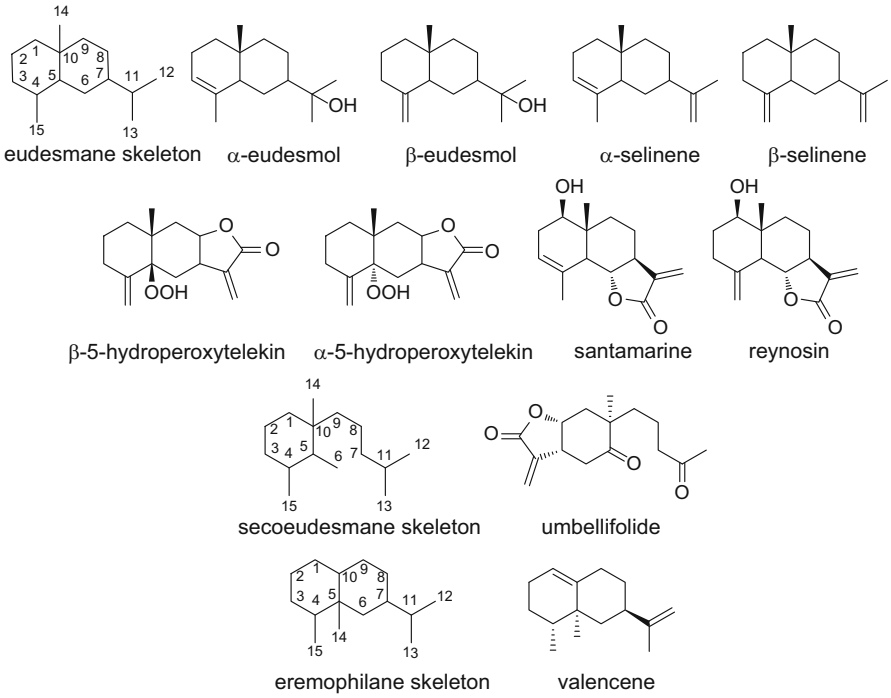


Fig. 21 Eudesmane-type sesquiterpene skeleton (top left) and some interesting eudesmanes found in spices (top right). Secoeudesmane-type sesquiterpene skeleton (middle left) and one of the secoeudesmanes isolated from *A. umbelliformis* (middle right). Eremophilane carbon skeleton (bottom left) and an important compound of this family isolated from herbs (bottom right)

Table 3. β-Eudesmol is also one of the most important compounds in hop (*Humulus lupulus*) used in beer making (Kishimoto et al. 2005). α-Selinene and β-selinene (Fig. 21) are two of the major components of the vegetable mitsuba (Okuno et al. 2017). These compounds are analogous to eudesmol but they lack a hydroxyl group.

Hydroperoxyeudesmanes, such as telekin derivatives, are unusual in nature (Fig. 21). In addition, due to their oxidative nature, they should be avoided in food or drink. Both α- and β-5-hydroperoxytelekins are hydroperoxyeudesmanolides (the -lide suffix indicates a lactone ring fused to the structure) present in *Artemisia umbelliformis*, which is used to generate alcoholic drinks. Apart from these compounds, the secoeudesmane skeleton is also found in *A. umbelliformis*. The seco- prefix indicates a ring aperture in the principal structure (Fig. 21), and a wide variety of natural products have this motif (Cappelletti et al. 1986; Cheng et al. 2014). On the other hand, eremophilanes have the same carbon skeleton as eudesmane, but with a methyl group rearranged. Valencene is a representative compound of this family, and it is commonly isolated from sweet orange. Furthermore, the relevance of this compound as a food flavor has led to an important industrial and biotechnological production (Frohwitter et al. 2014). Valencene is a component of the volatile compounds of citrus fruits, and it is present in orange peel, pulp, and juice (Orav and Kann 2001).

15.2.2.6 Germacranes

Germacranes are the most abundant sesquiterpene-type compounds in herbs. There are different subgroups within the germacrane family, and these include furangermacranes (Chen et al. 2005) and norgermacranes (Nevalainen and Koskinen 2002). Recently, two furan derivatives were isolated from *Curcuma zedoaria* (Matsuda et al. 2001), namely, furanodienone and glechomanolide (Fig. 22). Nevertheless, the most common compounds in this class are simple germacranes. These are characterized by a ten-membered ring with an isopropyl group in C-7 and two methyl

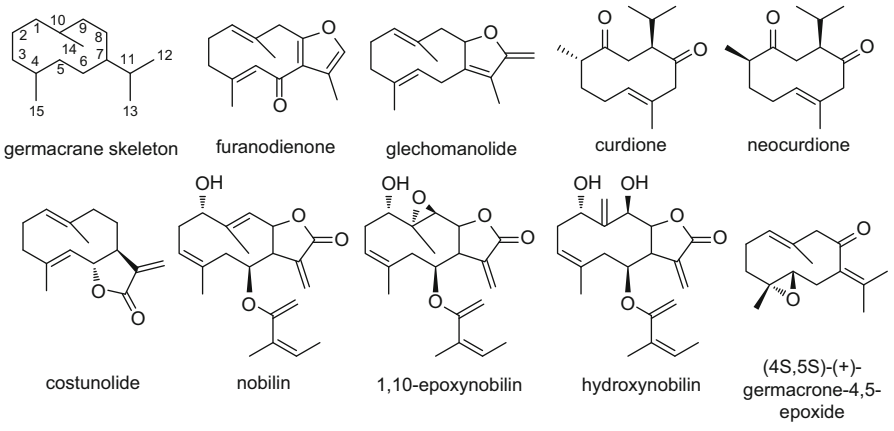


Fig. 22 Germacrane-type sesquiterpenoid (top left) and most relevant germacrenes in herbs

groups. Nobilin and its epoxy and hydroxyl derivatives are important germacrenes with antioxidant activity, and these can be isolated from chamomile extracts by supercritical fluid chromatography (Jones et al. 2014). Curdione and neocurdione (Fig. 22), as well as the previously mentioned curcumenol and isocurcumenol, were isolated from Zingiberaceae plants, and these two germacrenes have medicinal applications in cancer therapy. This kind of anticancer activity is found in costunolide, a well-known sesquiterpene lactone that can be isolated from *Artemisia umbelliformis*, which is used to distill Genepì liquor (Vouillamoz et al. 2015).

15.2.2.7 Other Structures

The sesquiterpenoids discussed above are the most relevant compounds in spices. Nevertheless, there are other sesquiterpenes with different types of carbon skeleton. The spatial arrangement is difficult to envision – for example, α-copaene found in laurel (*Laurus nobilis*) (Fig. 23) is a copaane with two fused six-membered rings and a twisted cyclobutane. This kind of crooked ring seems to be common, and examples include *trans*-α-bergamotene (bisabolane skeleton) found in basil oils or β-bourbonene (sesquiterpene-type bourbonane) found in *Satureja avromanica*, a spice used in Iran, *Mentha piperita*, and milk (Fernandez et al. 2003). More complex arrangements are uncommon, but they have an unexpectedly high stability, e.g., anisatin (Charonnat et al. 1996), a prezizaane found in the Japanese star anise (*Illicium anisatum*) (Ottesen and Magnuson 2010).

The total synthesis of α-copaene was achieved in 1992 by Wenker and co-workers. This synthesis starts with 5-isopropyl-2-methylphenol and involves more than 20 steps to obtain the target copaane (Wenkert et al. 1992). As mentioned

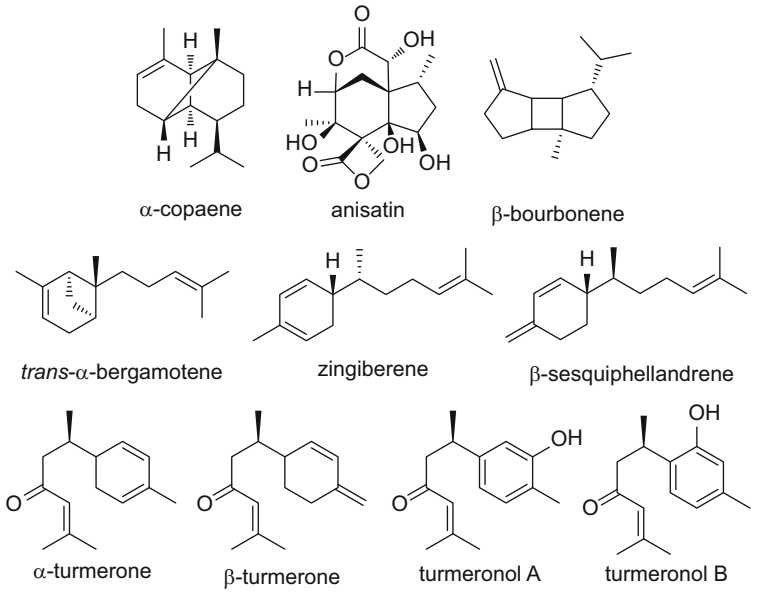


Fig. 23 Less common sesquiterpenoids found in herbs

previously, the syntheses of these natural products are not short or efficient. Another example is the β-bourbonene synthesis. Corbella et al. tried to prepare bourbonane by Beereboom's procedure, but this proved unsuccessful. However, in 1987 Kulkarni and co-workers devised a [2 + 2] photochemical cycloaddition as a key step to obtain the target sesquiterpene (Fig. 23) (Kulkarni et al. 1987).

Zingiberene and β-sesquiphellandrene (Fig. 23), both bisabolanes, are major sesquiterpenes found in ginger (*Zingiber officinale*), and they are important constituents of this spice (Govindarajan and Connell 1983). Some bisabolanes have also been isolated from fruiting bodies of the edible mushroom *Pleurotus eryngii*, which is endemic in Mediterranean areas, Central and Southern Europe, and Central and Western Asia (Kikuchi et al. 2018).

Rhizomes of curcumin are a source of turmeric, one of the most common spices used in cooking. Among turmeric constituents, the major components found in rhizomes are sesquiterpenes, and these impart aromatic properties. Four important sesquiterpenes have been identified: α-turmerone, β-turmerone, turmeronol A, and turmeronol B (Fig. 23) (Gupta et al. 2013).

Cedranes (Fig. 24) are less common sesquiterpenes in food, and their skeleton provides molecules with several types of biological activity (Tajabadi et al. 2018). Traces of cedrene have been identified as the volatile compound of sweet basil (Nurzyńska-Wierdak 2012). α-Cedrene epoxide has been reported as one of the main compounds in the mushroom *L. hatsudake* (Miyazawa et al. 2010).

L. hatsudake also produces a sesquiterpene with some similarity to cedrene, namely, the sesquiterpene-type clovane (Fig. 24) (Miyazawa et al. 2010).

Longifolanes (Fig. 24) are a family of tricyclic sesquiterpenes. Among these sesquiterpenoids, isolongifolanone can be found in certain foods such as mushrooms (*L. hatsudake*), in which is the major compound (Miyazawa et al. 2010). This product, which can be synthesized by oxidation of longifolene, allows the preparation of interesting derivatives that have shown positive fluorescence properties for sensing acidic pH (Wang et al. 2018).

Himachalenes (Fig. 24) contain a cyclohexane linked with a cycloheptane ring in positions 1 and 6. Like cedranes, these compounds are not representative families in food although traces of himachalol have been identified in some species of sweet basil (Nurzyńska-Wierdak 2012).

As shown above, a huge range of structures are present in spices, and it is difficult to envisage that only one kind of sesquiterpene could be present in any spice. On the contrary, it is common that numerous different types of sesquiterpene are present in the same spice.

In sweet basil many sesquiterpenes have been identified and quantified, with *trans*-α-bergamotene, germacrene D, and *epi*-α-cadinol being the major ones (Nurzyńska-Wierdak 2012). Bay leaf (*Laurus nobilis*) has a great wealth of sesquiterpenes (Table 3) (Peris and Blazquez 2015). In some species of sweet basil traces of the cadinenes, *cis*-muurola-4(14),5-diene and *cis*-muurola-3,5-diene (Nurzyńska-Wierdak 2012) have been isolated, and the latter compound has been also identified in the false truffle *Gautieria morchelliformis* (D'Auria et al. 2014).

Peppermint essential oil also provides β-farnesene, δ-cadinene, germacrene D, and germacrene-4-ol. δ-Cadinene is also present in sweet basil, orange juice, and orange soft drinks.

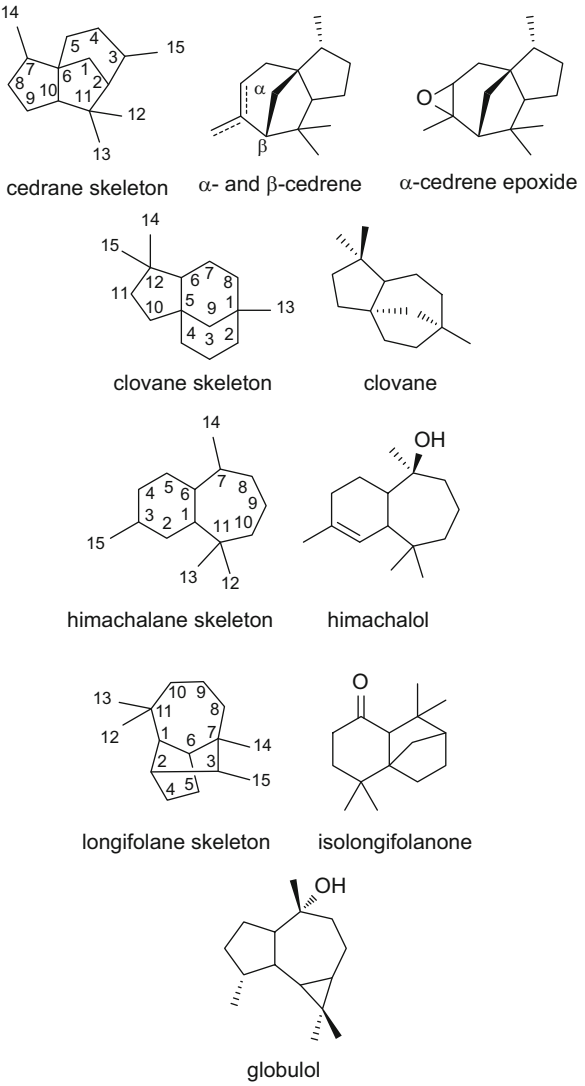
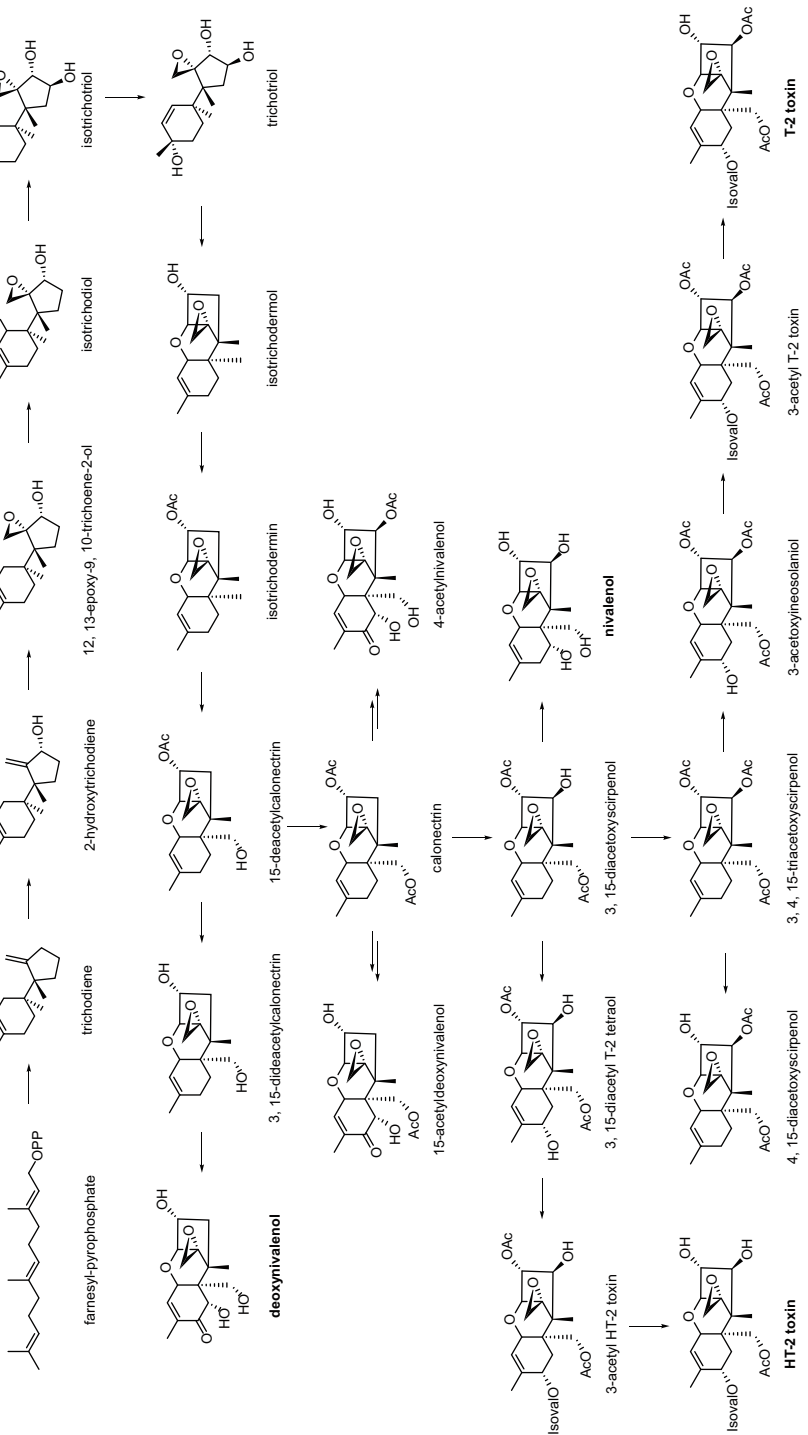


Fig. 24 Cedrane skeleton (top left), clovane skeleton (second line left), himachalane skeleton (third line left), longifolane skeleton (fourth line left), and some common structures of these families. Structure of globulol

15.2.3 Biosynthetic Route

15.2.3.1 Biosynthetic Route to Trichothecenes

The biosynthesis of trichothecenes (Scheme 3) starts from farnesyl pyrophosphate, which cycles into trichodiene. Trichodiene has been isolated from *Trichothecium roseum*, and different studies have suggested that this molecule is a precursor of the trichothecenes produced by this species. A monooxygenase is responsible for the transformation steps to obtain isotrichotriol from trichodiene.



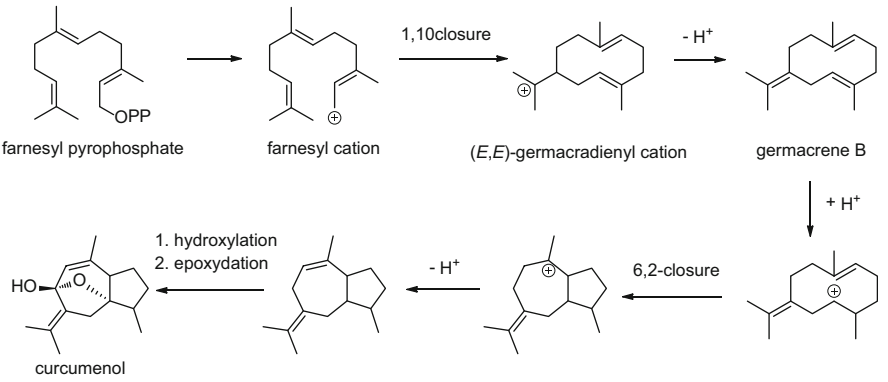
Scheme 3 Trichothecene biosynthesis. The molecules have been identified in different *Fusarium* species. Reproduced with the permission of (Foroud and Eudes 2009)

Numerous studies have been carried out to learn more about the biosynthetic pathway to trichothecenes. Among them, the treatment of strains of *Fusarium sporotrichioides* with oxygenase inhibitors should be highlighted (Desjardins et al. 1988) as this results in the inhibition of trichothecene production and an accumulation of trichodiene. This suggests that trichodiene is, indeed, a precursor of trichothecenes produced by *Fusarium* species. In the next steps, isotrichotriol undergoes two isomerization reactions. The first reaction involves the migration of the hydroxyl group from C-11 to C-9 to give trichotriol. The second reaction is a cyclization that occurs between C-11 and the oxygen atom located at C-2 to give isotrichodermol, which has the trichothecene skeleton structure. Isotrichodermol can also undergo an acetylation at C-3 and a subsequent hydroxylation at C-15 to produce 15-deacetylcalonectrin. From this point, 15-deacetylcalonectrin can follow different transformation pathways to give different trichothecenes. A hydroxylation at positions C-3 and C-7 and a ketone group addition at C-8 would produce DON.

Acetylation of 15-deacetylcalonectrin at C-15 leads to calonectrin, which is a substrate for other trichothecenes. Hydroxylation of calonectrin at C-4 produces 3,15-diacetoxyscirpenol, which can produce nivalenol, T-2 and HT-2 toxins. Nivalenol is produced by different hydroxylations and the addition of a ketone at C-8. T-2 and HT-2 toxin are obtained in different acetylation and hydroxylation reactions at C-3, C-4, and C-8 with subsequent addition of an isovalerate group at C-8. Concurrently, calonectrin and 3,15-diacetoxyscirpenol act as substrates for different deoxynivalenol and nivalenol derivatives.

15.2.3.2 Biosynthetic Route to Sesquiterpenes in Spices and Herbs

In the same way as for all sesquiterpenes, the first step involves farnesyl pyrophosphate, which undergoes two sigmatropic rearrangements to generate the guaiene skeleton (Kumeta and Ito 2010). In the case of curcumenol, a 6,2-closure reaction takes place, and a wide range of synthetic possibilities arise, with the emergence of functional groups. Nevertheless, a 1,10-closure step can give rise to different sesquiterpene skeletons, such as daucane or germacrenes (Scheme 4). Cadinanes are



Scheme 4 Biosynthetic pathway for curcumenol (guaiene)

a special case, and a sesquiterpene synthase has recently been discovered that belongs to the terpene synthase-a (TPS-a) clade and this catalyzes the formation of mainly cadinanes. This discovery was made possible by RNA sequencing to profile the transcriptome of *Laurus nobilis*. This plant, otherwise known as bay laurel, contains a broad range of sesquiterpenes: δ-elemene, β-elemene, β-caryophyllene, germacrene D, and α-bisabolene (Yahyaa et al. 2015).

Labeling experiments conducted with deuterium oxide showed that cadinane sesquiterpenes, such as cadalene, are mainly produced by protonation of the neutral intermediate germacrene D. This finding, which was developed by Garms and co-workers, provides an alternative pathway to the generally accepted one, which involves nerolidyl phosphate (Garms et al. 2010).

15.2.4 Analysis Techniques

15.2.4.1 High-Performance Liquid Chromatography (HPLC)

Trichothecenes can be detected without derivatization. However, the ultraviolet (UV) absorption of these compounds is weak, and detection is therefore difficult. Type B trichothecenes possess a conjugated enone system that enables their detection using a UV detector (Visconti and Bottalico 1983). Type A trichothecenes lack such a chromophore, and it is therefore necessary to use another type of detector, e.g., a refractive index detector (Schmidt and Dose 1984). DON has been detected in contaminated corn at levels of 5–10 ppb (Ehrlich et al. 1983). T-2 and HT-2 toxin were also detected by HPLC on rice samples (Schmidt 1981).

As stated above, in some cases detection of these compounds is difficult, and a derivatization step is required prior to analysis. Trichothecenes contain numerous hydroxyl groups, and these can be transformed into other groups with a strong UV absorption.

Trichothecenes can be treated with *p*-nitrobenzoyl chloride in the presence of 4-dimethylaminopyridine (4-DMAP) to obtain the corresponding trichothecene *p*-nitrobenzoate derivatives, which can be detected at 254 nm (Utley and Maycock 1985). Another procedure involves esterification of the hydroxyl groups using coumarin-3-carbonyl chloride, with the resulting trichothecene derivatives detected using a fluorescence spectrophotometer (Cohen and Boutin-Muma 1992).

Notwithstanding, the analysis of trichothecenes by HPLC is most commonly carried out with a mass detection system, and the most commonly reported are thermospray (Rajakylä et al. 1987) and fast atom bombardment (Kostiainen 1991).

15.2.4.2 Supercritical Fluid Chromatography (SFC)

Supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) allows the detection of trichothecene mixtures using supercritical CO at 100 °C as the mobile phase, without a derivatization step (Kalinoski et al. 1986; Roach et al. 1989; Smith et al. 1985). Both positive chemical ionization and negative ion chemical ionization were studied.

A SFC-MS/MS technique in which ammonia is used for chemical ionization allowed the separation of a DON, DAS, and T-2 toxin mixture with a picogram-range detection limit (Smith et al. 1985). In other studies this technique was used to detect DAS and T-2 toxin at ppm levels in wheat samples (Kalinoski et al. 1986).

Nobilin and 1,10-epoxynobilin have also been separated by supercritical fluid chromatography. Columns with sub-2 μm particles were employed because a smaller particle size stationary phase provides a reduced analysis time and increases the resolution and sensitivity. The column temperature was maintained at 50 °C and the temperature of the sample was 15 °C. The mobile phase employed to separate these two germacranes consisted of CO_2 as solvent A and methanol/isopropanol (1:1) as solvent B (Jones et al. 2014).

15.2.4.3 Gas Chromatography (GC)

The boiling points of sesquiterpenes are relatively low, and a gas chromatograph linked to a mass spectrometer is one of the most common systems used to identify sesquiterpenes (as well as other natural products like alcohols or aldehydes) in volatile oils isolated from different parts of the plant. Identification is achieved by comparison of mass patterns and retention indices (RI) with library data. A flame ionization detector (FID), which provides high sensitivity and low background noise, is the usual detector employed for these analyses.

Gas chromatography is the most widely applied method for the analysis of trichothecenes. Analysis of trichothecenes without derivatization is possible (Stahr et al. 1979), and different detectors can be used, with the electron capture detector (ECD) and mass detection being the most common.

Analysis of non-derivatized trichothecenes using oxygen negative chemical ionization gas chromatography/mass spectroscopy permitted the detection of DON, DAS, T-2 toxin, and HT-2 toxin among other trichothecenes in different food samples. The detection limit reached picogram levels (Miles and Gurprasad 1985).

Despite the fact that direct analysis of trichothecenes without derivatization is possible, most gas chromatography methods for the determination of trichothecenes in food include the derivatization of the hydroxyl groups of the molecules. The derivatization step increases the volatility of the sample and the sensitivity of the analysis.

There are two main derivatization processes used in the analysis of trichothecenes: the first involves silylation of the hydroxyl groups to form the trimethylsilyl (TMS) ethers (Ikediobi et al. 1971) and the other is the formation of fluoroacyl derivatives (Seidel et al. 1993).

Different detectors can be used in the analysis of trichothecenes, but the most common ones are an ECD or a mass detector.

ECD is a very sensitive and selective detector for trichothecenes (Kuroda et al. 1979). The conjugated carbonyl group of type B trichothecenes makes them sensitive to ECD, while for type A trichothecenes, formation of the fluoroacylation derivatives is recommended to obtain a greater sensitivity (Croteau et al. 1994).

Different mass detectors can be used in combination with gas chromatography, and the choice depends on the particular objective. The electron impact detector has

a detection limit of 0.1–1 ng, but the EI spectra provide limited structural information. Positive chemical ionization (PCI) analysis is more useful for qualitative identification (Mirocha et al. 1986) since it provides fewer fragments, and the more distinct ones allow the identification of the different trichothecenes. Negative ion chemical ionization (NICI) has also been applied in the determination of trichothecenes, and a higher sensitivity has been reached with this technique, which allows detection limits of femtograms to be achieved (Krishnamurthyt et al. 1986).

15.2.4.4 Countercurrent Chromatography (CCC)

Elemanes, and particularly β-elemene, have been selectively separated by countercurrent chromatography. The separation was carried out on the crude oil of *Curcumae Rhizoma*. Silver ions were employed to generate coordination complexes, which facilitate a better separation procedure. The selected solvent system was n-hexane/methanol/0.15 M silver nitrate (2:1.5:0.5 v/v/v). The results showed that β-elemene was eluted with other impurities, and silica gel column chromatography was therefore employed to remove them before CCC was applied. A total of 145 mg of β-elemene with >99% purity was obtained from 445 mg of partially purified β-elemene (Lu et al. 2017).

Costunolide has a similar polarity to another sesquiterpene in *Aucklandia lappa* and *Saussurea lappa*, so this compound is difficult to isolate and purify. Nevertheless, Li et al. developed a new method in high-speed countercurrent chromatography techniques to separate these components. The separation conditions provided a difference in retention time of more than 20 minutes between the compounds. 35.7 mg of costunolide and 43.6 mg of dehydrocostuslactone with 100% purity were isolated from 110 mg of crude product on using light petroleum/methanol/water (5:6.5:3.5 v/v/v) as the two-phase solvent system (Li et al. 2005).

15.3 Bioavailability and Metabolism

The incorporation of sesquiterpenoids in the human metabolic system can be difficult due to the solubility and bioavailability of the compounds. These terpenoids must fulfill certain physicochemical requirements before exercising their action, for example, as medicinal or antioxidant agents. Absorption, distribution, metabolism, and excretion (ADME) are the physicochemical aspects in question, and they also encompass solubility, lipophilicity, stability, and acid-base character. When a given sesquiterpene shows acceptable values for these characteristics, then the bioactive action will occur (Durán et al. 2018).

As mentioned above, solubility in water is an important factor in sesquiterpene metabolism. However, due to the wide variety of structures of sesquiterpenes, the presence of different functional groups that enhance solubility is frequent. These groups include hydrogen bond acceptors such as aldehyde or hydroxyl groups. The increase in solubility also improves the bioavailability in aqueous media, which is

the principal medium in the human body. Nevertheless, these groups are responsive to attack by acids in protic media, e.g., the stomach medium.

15.3.1 Bioavailability and Metabolism of Trichothecenes

Several studies have been performed to gain information on the metabolism of trichothecenes in mammals. These compounds are usually metabolized rapidly following different transformation paths. There are four main routes for metabolization. The first three are hydrolysis (Bauer et al. 1985; Corley et al. 1986), conjugation with glucuronic acid (Corley et al. 1985; Gareis et al. 1986), and hydroxylation (Knupp et al. 1987; Yoshizawa et al. 1985). These routes produce a wide variety of metabolites from trichothecenes, and these are less toxic than the original trichothecene.

The fourth metabolization route, and the one believed to represent a true detoxification, is the de-epoxidation of the epoxy group at C-12 and C-13. De-epoxy derivatives of DON and NIV are 54 and 55 times less toxic than DON and NIV, respectively. Some animals, including ruminants and rats, are able to reduce the epoxide group of the trichothecenes and subsequently remove them without suffering damage. Some research results have shown that the rumen microflora is able to reduce the epoxide group of the trichothecenes (Chatterjee et al. 1986; Swanson et al. 1987a) to give de-epoxy derivatives. Other studies have shown that rats excrete a high percentage of orally ingested trichothecenes as their de-epoxy derivatives (Sakamoto et al. 1986; Swanson et al. 1988).

15.3.1.1 T-2 Toxin and HT-2 Toxin

Since T-2 toxin is quickly metabolized into HT-2 toxin and their toxicity is similar, they are discussed together.

After ingestion, T-2 toxin is quickly absorbed and it is evenly transported in the organism. Tritium-labeled T-2 toxin was intravenously injected into swine, and 4 hours later 15–24% of the labeled toxin was found in the gastrointestinal tract. Approximately 5% of the toxins were found in other tissues, mainly the muscle and liver (Corley et al. 1986).

The main metabolization pathway that T-2 toxin follows is a quick deacetylation in position C-4 leading to HT-2 toxin. This reaction takes place mainly in the liver and it is catalyzed by a non-specific carboxyesterase (Johnsen 1988). HT-2 toxin is then further metabolized by different pathways and eliminated. T-2 toxin metabolite products are excreted via urine and feces. Depending on the species, differences in the proportions were observed (Eriksen and Alexander 1998).

15.3.1.2 Deoxynivalenol

DON in plasma is quickly and evenly distributed in the organism. DON was detected in many tissues 30 minutes after its intravenous injection in swine. The highest levels were detected in the liver and kidney, but lower concentrations were also found in many other tissues, including the brain, testes, heart, intestine, and spleen (Prelusky and Trenholm 1991).

DON can follow different metabolization pathways. However, the de-epoxidation to form DOM-1 is the most interesting pathway, as the toxicity of DOM-1 is much lower than that of DON. DOM-1 is a common metabolization product in rats (Yoshizawa et al. 1983). Cow rumen is able to reduce the epoxy group of DON to give DOM-1 (Côté et al. 1986). In this study, most DOM-1 was excreted in urine and feces. It is important to highlight that small residues of DOM-1 were found in the milk produced by cows that were fed with DON contaminated food.

15.3.1.3 Nivalenol

A diet rich in nivalenol was employed to feed swine, and the detection of the toxin in blood was achieved only 20 minutes after ingestion, thus showing a fast absorption. However, this absorption was prolonged, with an absorption from 11% to 43% observed during the first 7.5 hours after feeding. After 16 hours NIV was still being absorbed by the intestine. In the case of excretion, during the first week, NIV was excreted in feces without detecting any metabolization derivates (Hedman et al. 1997). However, in a similar study, 1 week after starting a diet rich in NIV, almost all of the NIV ingested was found in the feces of swine, and this was in the de-epoxy form (Hedman and Pettersson 1997).

Similar results were obtained with rats. A total of 80% of the ingested NIV was excreted in feces as de-epoxy NIV, while only 7% was detected unmetabolized (Onji et al. 1989).

15.3.1.4 Diacetoxyscirpenol

DAS was orally administrated to rats and mice, and the results showed that both species eliminated approximately 90% of the administrated dose through urine and feces during the first day. After 24 hours, low levels of DAS were absorbed in the organism, mainly in the gastrointestinal tract (Wang et al. 1990).

In other studies, rats produced several DAS metabolite derivatives upon ingestion. They were the results of hydroxylation and de-epoxidation reactions of DAS. In urine, 15-MAS, SCP, DE 15-MAS, and DE SCP were found at levels of 3.5%, 4.9%, 9.5%, and 7.2% of the administered dose, respectively. However, in feces only DE 15-MAS and DE SCP were found, with 9.5% and 18.9% of the administered dose, respectively (Sakamoto et al. 1986).

15.3.2 Bioavailability and Metabolism of Sesquiterpenes in Spices and Herbs

Drimane derivatives such as polygodial, warburganal, and ugandensidial (Fig. 18) found in the herb *Warburgia ugandensis* act as –SH acceptors for sulfhydryl groups in chemoreceptor membranes or with L-cysteine to give their usual hot taste. Thus, only the functionality and stereochemistry of these drimanes are necessary for the spicy flavor (Asakawa et al. 2012; Kubo and Ganjian 1981).

Some guaiane-type sesquiterpenoids also show a high level of hydrogen bond acceptor positions due to their multi-hydroxyl functionalization. Curcumenol, isocurcumenol, and procurcumenol (Fig. 20) are the guaianes present in *Curcuma*

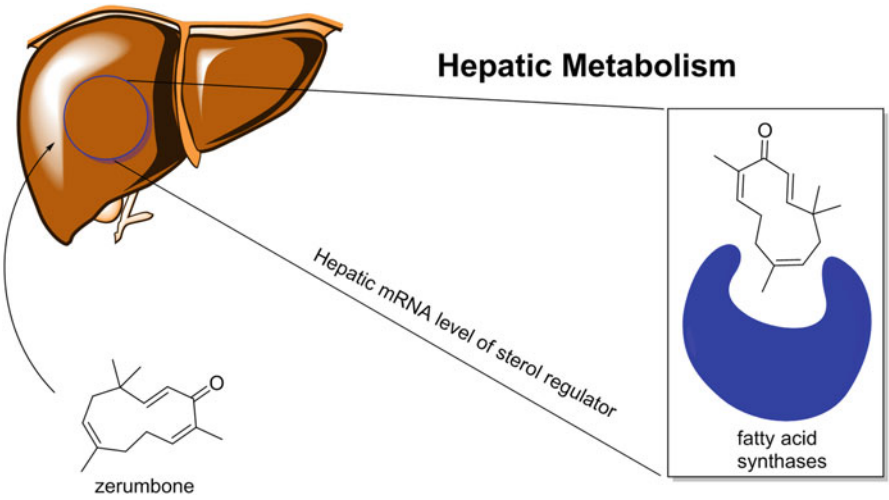


Fig. 25 Mode of action of zerumbone in liver

zedoaria, and they show diuretic and stimulant activity after being eaten (Lo et al. 2015). Curcumenol and its derivatives are nitric oxide (NO) suppressors. This inorganic free radical is produced by oxidation of L-arginine by nitric oxide synthase, which is involved in physiological processes as an inflammatory agent (Lo et al. 2015; Matsuda et al. 2001).

Humulene compounds show a complex metabolism because of their hepatic accumulation. Zerumbone interacts with lipogenic target genes, such as fatty acid synthases, acetyl-CoA carboxylase 1, and stearoyl-CoA desaturase-1. This results in an improvement in insulin resistance and the repression of hepatic lipogenesis (Fig. 25).

The bioavailability of sesquiterpenes and every natural product in the human body is conditioned by pharmacokinetic parameters such as clearance (volume of plasma from which a substance is completely removed per unit time), volume of distribution, or half-life time. Comparison of two usual sesquiterpenes found in food, namely, costunolide and dehydrocostuslactone (Figs. 20 and 22), showed that the bioavailability of costunolide is higher than that of dehydrocostuslactone, with a maximum plasma concentration of >12 μg/mL. Nevertheless, the clearance and the volume of distribution of dehydrocostuslactone are higher than those of costunolide. The persistence of these sesquiterpene lactones in metabolism is very small, with a half-life time of less than 2 minutes, and Peng et al. identified the degradation metabolites in the body, which can be explained by phase I biotransformation and phase II biotransformation (Peng et al. 2014) (Fig. 26).

In addition to metabolism, the way in which sesquiterpenes are consumed is a relevant factor in the degradation of these compounds. Biodegradation of sesquiterpenes in the aqueous environment is limited, and persistence in the human body can be a significant problem. Nevertheless, continuous exposure to aqueous media

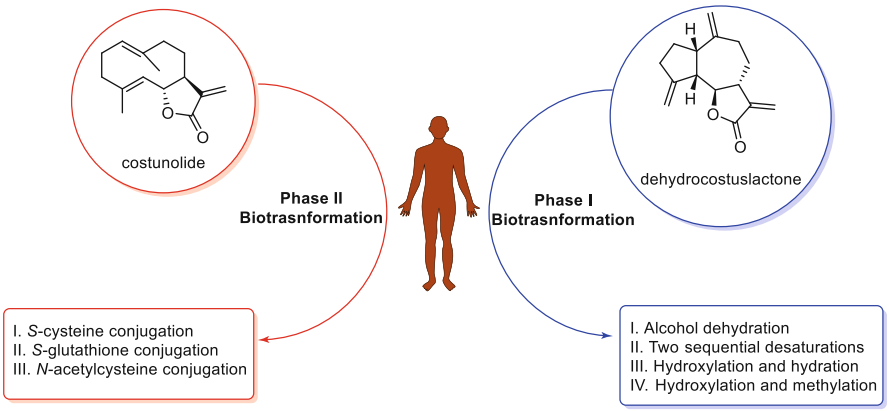


Fig. 26 Metabolism of costunolide and dehydrocostuslactone

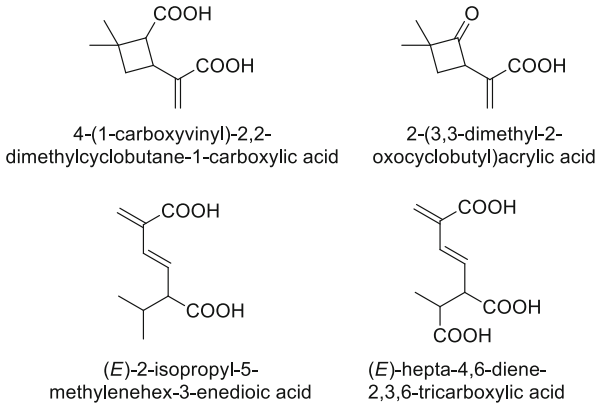


Fig. 27 Potential stable metabolites of β-caryophyllene (top) and germacrene D (bottom)

causes degradation of most sesquiterpenoids. Recent studies on stable metabolites associated with this degradation process have been carried out. β-Caryophyllene is degraded to cyclobutane carboxylic acids, while germacrene D is degraded to two hexadienes (Fig. 27) (Jenner et al. 2011).

It is also worth highlighting the possibility of post-oxidation, which leads to several kinds of oxygenated sesquiterpenes. This is the case for α-guaiene autoxidation derivatives (Fig. 28): α-epoxide and β-epoxide, 7-epi-chabrolidione, corymbolone, and rotundone (Huang et al. 2015).

As stated previously in this section, solubility plays an important role in the metabolism of sesquiterpenes, and this can be improved by different methods, such as encapsulation. It is possible to cover a sparingly soluble compound with a molecular structure that shows higher water solubility. The physicochemical properties of the encapsulated molecule are not altered by intermolecular interaction with the capsule. After being delivered, the encapsulated compound will act in the target

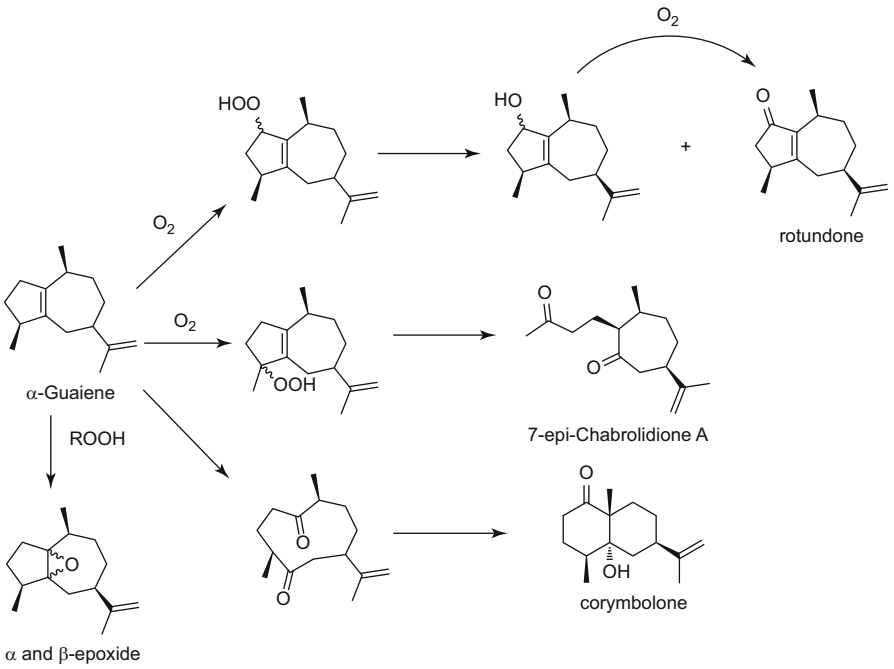


Fig. 28 Mechanistic of autoxidation of α-guaiene. Reproduced (adapted) with permission of Huang et al. (2015). Copyright 2015 American Chemical Society.

without the need to be structurally modified. This technique is usually applied in food, as in the case of β-carotene (vitamin A precursor), by the creation of supramolecular polymer micelles (Mejías et al. 2018a). Furthermore, this approach has been applied recently with sesquiterpene mimics with a polymeric core/shell system, where the solubility was enhanced by more than 27 times (Mejías et al. 2018b).

15.4 Bioactivities

15.4.1 Studies on Animals with Trichothecenes

Trichothecenes are capable of producing a wide range of effects and symptoms that are determined by the dosage of the mycotoxin and the duration of the exposure. Due to their fast metabolization, the symptoms produced by trichothecene poisoning appear quickly.

Several studies have been carried out to research the effect of trichothecenes on animals in their intake or intravascular administration. The authors concluded that these products show counterproductive effects (Côté et al. 1984; D'Mello et al. 1999). The most common effects are diarrhea, emesis, feed refusal, weight loss, gastrointestinal lesions, and, in the most extreme cases, death. However, besides

these common effects, trichothecenes are also responsible for many other dangerous effects. They can inhibit protein synthesis to produce alterations in the circulatory, reproductive, nervous, and immune systems, and they also have carcinogenic and dermal effects and genotoxicity, inhibit the synthesis of DNA and RNA, and produce modifications of the membrane structure (Eriksen and Alexander 1998; Corrier 1991).

15.4.1.1 Inhibition of Protein Synthesis

Some trichothecenes show a potent inhibition of protein synthesis activity (Wei et al. 1974). The mechanism of action starts through binding with the 60S subunit of eukaryotic ribosomes. They are able to inhibit the peptidyl transferase activity and the chain elongation of the protein synthesis process. There are different modes of action: some trichothecenes, like trichodermin and T-2 toxin, seem to reduce the activity of the peptidyl transferase required for the termination step (Warren 1973). On the other hand, other studies have demonstrated that T-2 toxin inhibits the initiation step, probably preventing the second amino acid from joining the first stage of the sequence (Eriksen and Alexander 1998). DON and NIV also showed protein synthesis inhibition. The mechanism of action for DON involves inhibition of the chain elongation (Ehrlich and Daigle 1987), while NIV inhibits initiation (Cundliffe and Davies 1977).

15.4.1.2 Inhibition of DNA/RNA Synthesis

Numerous studies have shown how trichothecenes inhibit the incorporation of thymidine and leucine in the DNA strand. For example, HeLa cells were treated with different concentrations of NIV, and cell proliferation was inhibited at concentration of 0.5 μg/mL. In this study, the incorporation of tritium-labeled thymidine, leucine, and uridine into DNA or RNA was measured. The results showed that at a concentration of 50 μg/mL, thymidine and leucine incorporation was almost completely inhibited. Notwithstanding, uridine incorporation only experienced 10–15% inhibition at the same concentration (Ohtsubo et al. 1968).

T-2 toxin also inhibited the incorporation of thymidine into DNA when it was tested on thymus cell lines (Munsch and Müller 1980). A slight inhibition was observed for concentrations from 0.1 to 1 ng/mL. When the concentration was greater than 10 ng/mL, high levels of inhibition were reached. The effect of T-2 toxin and DAS in human peripheral blood lymphocytes was studied by analyzing the incorporation of labeled tritiated thymidine in DNA synthesis. An inhibition of 50% was reached with concentrations of 1.5 ng/mL of T-2 toxin and 2.7 ng/mL of DAS. Total inhibition was achieved with 8 ng/mL of both toxins (Cooray 1984). Furthermore, DON is able to inhibit completely the incorporation of thymidine and leucine in mice spleen and Peyer's patch cells at a concentration of 1000 ng/mL (Warner et al. 1994).

15.4.1.3 Effects on the Immune System

Trichothecenes have an important and broad immunosuppressive activity. Many studies have shown that T-2 toxin causes necrosis and lymphoid depletion in the

spleen, thymus, and lymphoid nodes in some animals like sheep (Friend et al. 1983), pig (DeNicola et al. 1978), and chicken (Wyatt et al. 1973). Other trichothecenes like DAS and DON have proven to have similar but less marked effects (Côté et al. 1985; Lafarge-Frayssinet 1979).

T-2 toxin (Buening et al. 1982), DAS (Lafarge-Frayssinet 1979), and DON (Atkinson and Miller 1984) reduce the blastogenic response of lymphocytes to mitogens. In the studies performed by Lafarge-Frayssinet and co-workers (Lafarge-Frayssinet 1979), T-2 toxin increased the lymphocyte B and T response at low concentrations. However, it showed a strong inhibition at higher concentrations.

Trichothecenes could also provoke alterations in the immunoglobulin level regulation system. When cows were administered with T-2 toxin, their concentration of immunoglobulins A (IgA) and M (IgM) showed lower levels compared with the control. Immunoglobulin G (IgG) did not show any response to the toxin (Mann 1982). Mice fed with DON showed an increase of IgA levels, but IgM and IgG levels decreased (Pestka et al. 1989).

Besides the aforementioned activities, trichothecenes have many other effects on the immune system. If a living being is intoxicated with trichothecenes, they will have more susceptibility to infectious diseases. T-2 toxin decreases the resistance to salmonellosis (Tai et al. 1988) and tuberculosis (Kanai and Kondo 1984) in mice. In addition, mice intoxicated with DON showed less resistance to *Listeria monocytogenes* infection (Tryphonas 1986) and *Salmonella enteritidis* (Hara-Kudo et al. 1996).

There have also been cases where trichothecenes have increased resistance to bacteria. For example, mice infected with *Staphylococcus hyicus* and *Mycobacterium avium* were given DON and T-2 toxin orally. The results showed a reduction of infection from both bacteria tested (Atroshi et al. 1994).

15.4.2 Studies on Animals with Sesquiterpenes Found in Spices and Herbs

Activities shown by sesquiterpenoids are focused in seven specific types: antioxidant, antibacterial, antihyperlipidemic, antihyperglycemic, anti-inflammatory, neuronal, and circulatory system alterations and anticancer activities. Antioxidant properties are mainly connected with the presence of double bonds and heteroelements with electron acceptor capability. Antibacterial activity is explained as being due to high lipophilicity that allows them to cross the bacteria membrane toward cytosol action. Antihyperlipidemic and antihyperglycemic effects correspond with liver accumulation as a first barrier in the human body. The capability for prostaglandin inhibition allows these compounds to act as anti-inflammatory agents. Their neuronal system effects are believed to be produced by their ability to suppress efferent adrenal sympathetic nerve activity and modifications to intracellular ionic calcium (Ca^{2+}) concentrations. Some studies have also demonstrated that certain sesquiterpenes inhibit the proliferation of cancerous cells.

15.4.2.1 Antioxidant Activity

Antioxidant activity does not seem to have a correlation with compound skeleton type, but a high number of double bonds are present in all of the compounds that show this bioactivity. Spathulenol and α-bulnesene have a similar sesquiterpene structures, with seven-/five-membered rings, and they are the sesquiterpenes in spices with better profiles for this activity. Two experiments to study antioxidant capability have been carried out, namely, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect and decreased levels of thiobarbituric acid reactive substances. In terms of reactive oxygen species (ROS) scavenging, β-caryophyllene modulates thermal and oxidative stress, thus decreasing intracellular free radical amounts and maintaining the intracellular ROS equilibrium with redox homeostasis (Pant et al. 2014).

In the case of the 2,2-diphenyl-1-picrylhydrazyl radical, the reaction of this compound with the bioactive agent was studied, and the loss of optical density was evaluated to verify the antioxidant power (Nenov et al. 2011). Spathulenol was tested with this method after being extracted from cinnamon (*Cinnamomum zeylanicum*). α-Bulnesene, isolated from *Ocimum sanctum* – commonly known as holy basil – also shows antioxidant activity. This activity showed a notorious inhibition of the thiobarbituric acid reactive species in mouse blood (Suanarunsawat et al. 2010) (Fig. 29). This kind of bioassay determines the presence of the three antioxidant enzymes: glutathione peroxidase, catalase, and superoxide dismutase. If the cell shows low levels of these enzymes, the oxidative stress is also low, and the compound consumed, α-bulnesene, has antioxidant properties. Low levels of oxidative stress avoid the production of antioxidant enzymes by the cell (Kaur et al. 2012). Furthermore, in vitro studies with sesquiterpenoids showed that β-caryophyllene modulates the lifespan of the soil nematode *Caenorhabditis elegans*. These bacteria are used as a model for aging studies, and the results indicated an increase in the lifespan value by over 22% at a dose of 50 μM.

15.4.2.2 Antibacterial Activity

Despite the antioxidant activity of β-caryophyllene, this molecule stands out due to its antibacterial activity. β-Caryophyllene can be found in mint (*Mentha* spp.) (Park et al. 2016), in savory (*Satureja avromanica*) (Abdali et al. 2017), in lime peel (*Citrus aurantifolia*) (Jafari et al. 2011), and in cinnamon extracts (*C. zeylanicum*) (Nenov et al. 2011). The most common method to evaluate the antimicrobial/antibacterial activity is the disc diffusion assay. Different bacterial cultures, including *Escherichia coli* (*E. coli*), *Aeromonas salmonicida*, *Bacillus subtilis*, and *Staphylococcus aureus*, are swabbed onto agar plates overnight. A solution with a concentration of around 30 μL is then added to the Whatman disc, and the sample is incubated overnight at 37 °C. The inhibition zone is measured and this provides an indication of the antibacterial activity (Balachandran et al. 2015). Cubenol, as an isolated compound, is also strongly active against *E. coli*.

Sesquiterpenes tested in antibacterial tests are usually assayed as extracts, such as β-bourbonene or *trans*-α-bergamotene (Abdali et al. 2017). One problem associated

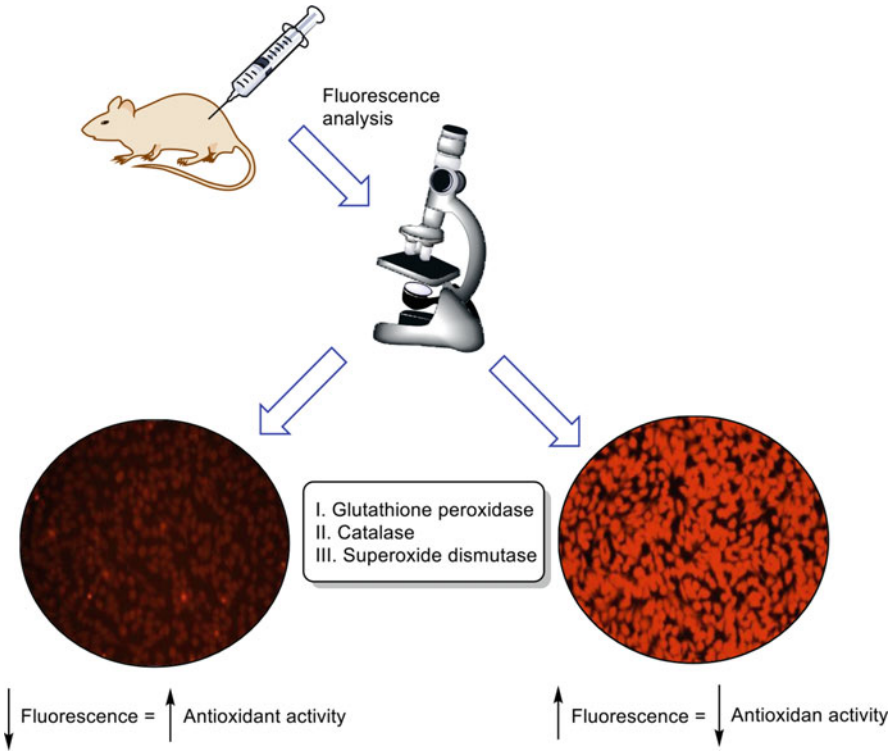


Fig. 29 Scheme of antioxidant assay in mouse blood by quantification of enzymes related with thiobarbituric acid reactive species

with extract tests is the potential for joint action of sesquiterpenes. In industry it is common to apply pure extracts of compounds, but an in-depth analysis of the active ingredients provides information on toxicity and side effects. The removal of unnecessary secondary compounds from the extracts may lead to cost reductions in manufacturing. Synergism can become a complex issue when mixtures are not binary, such as cadinane and caryophyllene sesquiterpenes in *Thymus saturejoides* (Kasrati et al. 2014).

Basil essential oil (*Ocimum basilicum*) has a strong antibacterial activity, as demonstrated by in vitro tests against Gram-positive bacteria *Enterococcus faecalis*, *Micrococcus luteus*, *Sarcina* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus mutans* and Gram-negative bacteria *Acinetobacter* sp., *Aeromonas* sp., *Citrobacter freundii*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella choleraesuis*, *Serratia marcescens*, *Shigella flexneri*, and *Yersinia enterocolitica*. Minimum inhibitory concentrations can differ from 0.25 to 1.00 mg per gram for the bacteria listed above. *Pseudomonas aeruginosa* was the only sample tested that did not suffer inhibition (Gaio et al. 2015).

15.4.2.3 Antihyperlipidemic Activity

Antihyperlipidemic effects have been found in sesquiterpenes, and this is mainly due to liver accumulation of these compounds. Predominantly, antihyperlipidemic action is due to suppression of liver lipid synthesis (Suanarunsawat et al. 2010). In the cases of α-bulnesene and β-caryophyllene, their effectiveness is comparable to that of the reference drug simvastatin (Suanarunsawat et al. 2009). Notwithstanding, zerumbone is the sesquiterpene with the highest antihyperlipidemic activity. This humulene is usually isolated from ginger (*Zingiber zerumbet*), and it reduces cholesterol levels in plasma and triglycerides in hepatic tissue of hamsters. The liver of the animal is removed and extracted with chloroform/methanol (1:2) in order to analyze the lipids. After oral administration of zerumbone, fat accumulation in the liver was reduced, and, at the same time, insulin resistance was improved, inflammation was reduced, and hepatic lipogenesis was inhibited by repression of sterol regulatory element-binding protein 1 (SREBP-1c) (Tzeng et al. 2013, 2014). At plasma level, zerumbone has been tested for vasorelaxation on rat aorta, and it showed myorelaxation. Nevertheless, zerumbone does not present positive modulation of L-type Ca^{2+} channel influx, so K^+ channel opening activity may be involved.

15.4.2.4 Antihyperglycemic Activity

Antihyperglycemic studies on sesquiterpenes are carried out in diabetic rats and rabbits. Streptozotocin and alloxan are the compounds used to induce diabetes in animals (Fig. 30) by a single administration of the drug. In the case of rat, different effects were observed after diabetes induction: increased glucose, increased HbA1c, falls in insulin and hemoglobin, falls in liver and skeletal muscle glycogen, and alterations in glycogen synthase and phosphorylase activity. The continuous oral administration of β-caryophyllene significantly decreased the glucose level by

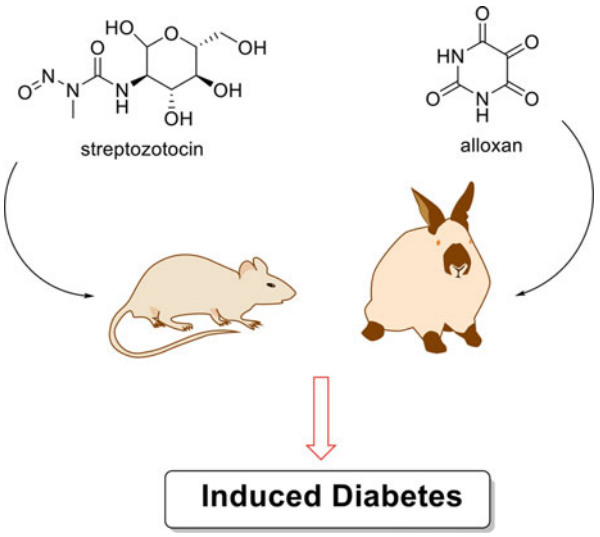


Fig. 30 Compounds used in the diabetic induction process

boosting insulin levels. After 45 days, the sesquiterpenoid restored glucose homeostasis by modulating the activity of enzymes involved in glucose utilization (Basha and Sankaranarayanan 2014). In the case of rabbit, β-caryophyllene was again tested, but it was studied as the extract from *Myrtus communis*, which has a high contribution from α-humulene. The level of glucose was determined by performing a standardized oral glucose tolerance test, and it was verified that sesquiterpenes in the essential oil of this spice influence the intestinal transport mechanism of carbohydrates. In particular, Sepici et al. stated that the extract of *M. communis* inhibits α-glucosidase of the small intestine, so glucose delivery is delayed (Ben Hsouna et al. 2014; Sepici et al. 2004).

15.4.2.5 Anti-inflammatory Activity

Among sesquiterpenes, α-guaiene (Fig. 19) is a promising candidate to replace current nonsteroidal anti-inflammatory drugs (NSAIDs), whose side effects are dangerous, despite their effectiveness in inhibiting prostaglandin synthesis (Tracey 2002). Prostaglandins are compounds that play an important role in the inflammatory response and they are synthesized by catalysis of the enzyme cyclooxygenase (COX) (Lipsky 1999). The results of in vitro bioassays show that α-guaiene inhibits COX-1 and COX-2 with half maximal inhibitory concentration (IC_{50}) values of 43 and 84 μg/mL, respectively. Even better results were obtained when this compound was tested in vitro against 5-lipoxygenase (IC_{50} of 27 μg/mL) and acetylcholinasterase (IC_{50} of 21 μg/mL) (Eldeen et al. 2016). In addition, 5-lipoxygenase works in the biosynthesis of the proinflammatory mediators the leukotrienes in collaboration with acetylcholinasterase, an enzyme that catalyzes the breakdown of acetylcholine, which is a parasympathetic neurotransmitter that inhibits the liberation of proinflammatory mediators (Borovikova et al. 2000).

15.4.2.6 Neuronal System Alterations

β-Eudesmol suppresses efferent adrenal sympathetic nerve activity (ASNA) in rats by intragastric administration. This task is carried out by the afferent vagus nerve and the calcium-permeable nonselective cation channel TRPA1 (expressed in the stomach). Plasma adrenalin levels decrease after the administration of β-eudesmol (Ohara et al. 2018). Mice neuronal outgrowth is also induced by β-eudesmol after increasing the intracellular Ca^{2+} concentration (Obara et al. 2002). More properties have been reported for this compound, and it is worth highlighting the propitious inhibition produced with *in vitro* and *in vivo* tests on angiogenesis, whose disorders contribute to cancer and diabetic retinopathy (Tsuneki et al. 2005). Oral administration of this sesquiterpene also influences the digestive system, as evidenced by the dose-dependent promotion of gastrointestinal motility in mice (Wang et al. 2002).

15.4.2.7 Effects on the Circulatory System

Ginger has been suggested as an alternative antithrombotic drug. Due to its traditional uses, this spice has been tested in human studies. Platelet thromboxane production was evaluated *in vivo*, and when 5 g of raw ginger were consumed daily by humans for 7 days, a significant reduction in mean thromboxane B2 (TxB_2)

to 37% was observed upon taking measurements in serum after blood clotting (Srivastava 1989). The synthesis of the precursor of this product is one of the main targets of aspirin. A subsequent study, in which TxB_2 was measured in human blood after 2 weeks of ginger ingestion, did not provide evidence of antithrombotic activity due to the lack of significant differences with subjects treated with placebo (Janssen et al. 1996). A similar finding was obtained with rats that had consumed ginger daily for 4 weeks, with insignificant reductions of TxB_2 levels. Nonetheless, two other benefits were demonstrated. These were significant changes in the serum prostaglandin-E_2 (PGE_2) at a dose of 50 mg/kg and a significant reduction of serum cholesterol, with the strongest effects obtained at the higher doses tested (500 mg/kg). At lower doses, serum cholesterol reduction was only produced when ginger administration was conducted intraperitoneally (Thomson et al. 2002).

15.4.2.8 Anticancer Activities

Anticancer *in vitro* activity has been suggested for β-caryophyllene and α-humulene, particularly against RAW264.7 (murine macrophage) and HCT-116 (colon cancer) cell line growth. Fractions with α-humulene showed higher activity than fractions rich in β-caryophyllene (El Hadri et al. 2010). Pharmacokinetics of α-humulene have been studied in mice. If this sesquiterpene is orally administered, it will take only 15 minutes to be detected in plasma, with the concentration decreasing thereafter and being undetectable after 12 hours. Upon intravenous administration, this period decreased to 2 hours. The half-lives are 16.8 minutes and 1.8 minutes, respectively. High concentrations of α-humulene were found in the liver, although it was also present in kidneys, heart, lungs, spleen, and brain (Chaves et al. 2008). Turmeric has been shown to have several types of activity. Different human studies have proven its anticancer activity, as well as activity against acne, diabetes, fibrosis, irritable bowel syndrome, and lupus nephritis. Turmeric has also shown potential in animal studies against atherosclerosis, depression, inflammatory processes, neurodegeneration, and obesity (Gupta et al. 2013).

15.5 Benefits

A small amount of research has been carried out in clinical studies on sesquiterpenes found in spices. Horváth et al. reported an interesting analysis concerning the relation between β-caryophyllene and damage reduction in liver after cisplatin anticancer applications.

The anti-inflammatory activity of this caryophyllane is already known, but it has recently been shown to be a natural agonist of endogenous cannabinoid 2 receptors, which are expressed in immune cells. These receptors are damaged after cisplatin treatment and they induce nephron toxicity. Therefore β-caryophyllene liver accumulation has tremendous therapeutic potential in cases involving treatment against cancer (Horvath et al. 2012).

Very little information about the possible benefits of trichothecenes for human health is available, mainly because of the numerous secondary effects. Even so, the diverse range of activities found for trichothecenes suggest that further studies could lead to a way to transform these toxic compounds into therapeutic agents. There are cases where mycotoxins (Shapira and Benhar 2010) are used for therapeutic purposes.

Deacetoxyscirpenol was investigated as a possible molecule to treat cancer in humans (Sudakin 2003). In Phase I clinical trials secondary effects were not found at low doses. Nevertheless, increases in the dose did cause several adverse effects, such as gastrointestinal lesions, among others. As a result, the study was stopped, and the possible medical applications did not go beyond the Phase II stage.

15.6 Application in Food

Food industries have a high demand for natural preservative compounds with additional benefits. Sesquiterpenes have shown this ability, and they have therefore attracted great interest for applications in foodstuffs to provide added value. In this respect it is worth noting the use of sesquiterpenoids as antioxidants, as preservatives (due to their antibacterial activity), and as a source of flavor or aroma.

In some cases, sesquiterpenes are used as natural food additives due to their antioxidant properties, and one example is the use of extracts obtained from eucalyptus leaves. Even though sesquiterpenes are not the strongest antioxidant compounds found in leaves, it is worth highlighting the presence of globulol (Fig. 24), as this is one of the major constituents, and other sesquiterpenes such as aromadendrene (Amakura et al. 2002).

Hydroperoxyeudesmanolides, which were mentioned in a previous section, show antioxidant activity, and they are isolated from *Artemisia umbelliformis*. The application of these compounds in Genepì liquor provides extra value to this drink. Distillation of the raw plant enables the solubilization of hydroperoxide derivatives from plant trichomes (Cappelletti et al. 1986).

During beer brewing, hops are added with the aim of providing bitterness and aromas. β-Eudesmol is a sesquiterpenoid produced by hop species, and it is a contributor to the spicy aroma of beer. In addition, β-caryophyllene, α-humulene, humulene epoxide I, and β-farnesene are compounds that contribute to the sensory properties of beer. Aromatic distinctions can be provided by other types of compound (terpenoids, ketones, acids, etc.), but the levels of many of them decrease during the wort boiling process. This technique is usually applied at the same time as the addition of hops. The previously mentioned sesquiterpenes show a slow linear decrease in concentration when they are subjected to boiling procedures (Kishimoto et al. 2005). Specific aromas are associated with oxygenated sesquiterpenoids from hop, e.g., 14-hydroxy-β-caryophyllene in cedar odor; caryophylla-4(12),8(13)-diene-5-ol (α and β) in lime, herbal, and rubber; (*3Z*)-caryophylla-3,8(13)diene-5-ol (α and β) in lime, herbal, and pine; humulenol II in sagebrush, lime, cedar, and pineapple; and humulene epoxide III in lime and cedar for spicy and rubber aromas (Praet et al. 2016).

However, the main applications of sesquiterpenes from spices are not in drinks but in pastries and cakes. Cream-filled cakes are mainly contaminated with *Staphylococcus epidermis* and *Bacillus subtilis*. The sesquiterpene components of lime essential oil have an antibacterial activity against these bacteria. The reduction in the use of synthetic preservatives such as salt, nitrites, and sulfites is growing due to a demand by consumers for natural products. In the lime extracts employed, β-caryophyllene and *trans*-α-bergamotene are the principal sesquiterpenoids that provide antibacterial action (Jafari et al. 2011).

The antimicrobial activity of black and green pepper essential oils has been tested to inhibit the growth of *Staphylococcus aureus* bacteria in chicken soup. Both peppers showed significant antimicrobial activity, especially the green pepper. As a consequence, these ingredients could be used as natural food preservatives (Nikolic et al. 2015). This is also the case for basil essential oil, which has shown positive effects in controlling *Staphylococcus aureus* proliferation in Italian-type sausage, with the highest activity reported over the first 14 days (Gaio et al. 2015).

Another example is α-cadinol, due to its inhibitory properties on anaerobic bacteria. This sesquiterpene could be included in food, chewing gums or dentifrices to prevent the growth of *Streptococcus mutans* (EP130027A2 1985), a bacterium in the human oral cavity that is associated with tooth decay.

Furthermore, (+)-valencene is another relevant sesquiterpenoid that is a constituent of essential oils of citrus. In this case, (+)-valencene has recently been studied in terms of its biotechnological production from farnesyl pyrophosphate by *Corynebacterium glutamicum*. In author's opinion, the industrial production of this compound with application as anti-proliferative cancer cells and as flavor in food could reduce the costs to be applied as future drug or food supplement (Zhu et al. 2011).

It was shown that simple heterologous expression of a (+)-valencene synthase gene does not allow *C. glutamicum* to produce the sesquiterpene in question. However, when endogenous prenyltransferases were replaced by farnesyl pyrophosphate synthase from *E. coli*, the heterologous expression of (+)-valence synthase genes did result in (+)-valencene production (Frohwitter et al. 2014).

The determination of the best date to initiate grape harvest is a complex task for oenologists. Their efforts are mainly focused on the determination of numerous parameters like acidity or sugar content. However, these parameters do not give sensorial information that is linked to the future organoleptic wine properties. Novel methods are being developed to overcome this problem and these include, for example, focusing on the identification and quantification of particular maturity markers. Thus, some sesquiterpenoids are appropriate for this task, such as bicyclogermacrene, a germacrane employed as a maturity marker for winemaking (Perestrelo et al. 2018).

15.7 Safety

Despite the fact that sesquiterpenes show impressive benefits, they also contain some compounds or derivatives that can be dangerous upon continuous exposure.

15.7.1 Toxicity and Side Effects of Trichothecenes

Very few *in vivo* studies have been carried out on the toxicity of trichothecenes in humans. However, in the last century, many intoxication outbreaks occurred. The most important was in the USSR (Union of Soviet Socialist Republics) in 1932–1947 when, under famine conditions, the population consumed grains contaminated with *Fusarium sporotrichioides* and *Fusarium poae*. Tens of thousands of people were affected and 60% mortality was reported. The intoxication produced alimentary toxic aleukemia (ATA), and four stages were observed in people depending on the exposure and acuity (Joffe 1986). In the first stage, nausea, vomiting, diarrhea, abdominal pain, and lesions in the gastrointestinal tract were detected. When the exposure to the toxins was prolonged, stage two of the disease would produce an improvement of the symptoms observed in the first stage, with the development of anemia, thrombocytopenia, and leukopenia. Continued ingestion of the toxins led to stage three, where necrosis lesions would appear in the gastrointestinal tract and infections and internal hemorrhages occurred.

Besides the aforementioned case, there have been other outbreaks of trichothecene intoxication – mainly in Japan, China, and India (Eriksen and Alexander 1998; Peraica et al. 1999). Nevertheless, the intensity of the symptoms and the impact on the population were much smaller than the case observed in the USSR.

In 1987 the consumption of bread prepared with trichothecene-contaminated wheat from *Fusarium* spp. in India led to the appearance of abdominal pain, throat irritation, diarrhea, and emesis among other symptoms. Studies on the bread demonstrated the presence of DON, NIV, and T-2 toxin among other trichothecene derivatives (Bhat et al. 1989).

The levels of trichothecenes in cereals are a major concern worldwide, and the Scientific Committee on Food established a TDI for some of these compounds. For DON, the TDI is 1 μg/kg of body weight/day. T-2 and HT-2 toxin have a combined temporary daily intake (t-TDI) of 0.06 μg/kg of body weight/day, and a t-TDI of 0.7 μg/kg of body weight/day was established for NIV.

Not many studies have been carried out on humans. However, these compounds seem to have the same effect in humans as in animal assays. DON inhibited both protein and DNA synthesis in human intestinal cells (Kouadio et al. 2005). DNA synthesis was also inhibited by T-2 toxin, DON, and DAS when tested on human lymphocytes. This produced an inhibition of lymphocyte proliferation (Atkinson and Miller 1984; Cooray 1984).

15.7.2 Toxicity and Side Effects of Sesquiterpenes Found in Spices and Herbs

Curcuma aromatica usually shows antioxidant (Dong et al. 2018), anti-angiogenic (Kim et al. 2002), and anticarcinogenic activities (Xiang et al. 2017), and it has recently been introduced as a dietary supplement in Japan. Nevertheless, (4*S*,5*S*)-(+)-germacrone-4,5-epoxide (Fig. 22) is also present in this spice, and this is a germacrane-type sesquiterpene that significantly inhibits human cytochrome P450

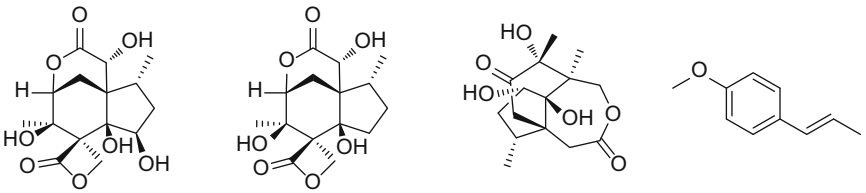


Fig. 31 Principal sesquiterpene toxins in Japanese star anise and the active ingredient in bird flu treatment (right)

(Bamba et al. 2011), which is involved in the oxidative phase of metabolism (Li et al. 2018b), and D_3 vitamin synthesis (Slominski et al. 2018). Furthermore, this effect has been seen with nerolidol and farnesol, which increase the inhibition of cytochromes when administered together (Spicakova et al. 2017).

A review of all previous sections makes clear the relevance of β-caryophyllene within the sesquiterpene groups. The presence of β-caryophyllene at higher levels than other sesquiterpenoids and its multiple bioactivities make it a good candidate for in-depth studies. Oliveira et al. developed an assay in mice with oral administration, and the results indicated an absence of toxicity in all internal organs and hematological aspects (Oliveira et al. 2018).

In relation to toxicity, Japanese star anise has led to controversy in different research investigations related to its benefits and adverse effects. *Illicium anisatum* (Japanese star anise) is usually employed in culinary applications as well as in infusions or tea. One of the sesquiterpene lactones found in this herb is anisatin (Fig. 31), a potent noncompetitive antagonist of γ-aminobutyric acid-dependent neurons in mammals. The receptor mentioned, and also the derivatives (neoanisatin and pseudoanisatin), is a ligand-gated ion channel responsible for important neurotransmission in the brain. The high toxicity of anisatin and its derivatives, like neoanisatin, can result in convulsant activity, hallucination, or epileptic (Ottesen and Magnuson 2010; Schachter 2009; Shen et al. 2012). On the other hand, Japanese star anise contains anethole, which is traditionally employed to cure rheumatism and is also is the active ingredient of bird flu treatment (D'Souza et al. 2017).

According to studies carried out on *I. anisatum*, it is difficult to conclude whether Japanese star anise is really a dangerous food. On analyzing the components present, prolonged exposure to anisatin and derivatives could become unsafe, but a limited administration as a spice is advantageous.

15.8 Marketed Products

15.8.1 Cereal-Based Food

Fusarium infection in cereal crops may occur when they are in the field or in storage. Numerous studies have demonstrated that the industrial processes involved in food preparation and production can reduce the presence of trichothecenes.

Notwithstanding, they cannot be completely removed, and so they are present in many cereal-based foods. Trichothecenes have been detected in soy-based, barley-based, oat-based, rice-based, corn-based cereals, biscuits (wheat based), maize, wheat flour, and other food like bread and beer (wheat beer).

15.8.2 Sesquiterpenes Marketed from Spices and Herbs

Most of the spices discussed in this chapter are marketed products that are available to anyone. Some of them are more accessible than others due to location and production. The principal sesquiterpenes analyzed in herbs that are consumed by humans are shown in Table 4.

Table 4 Marketed spices and their most relevant sesquiterpenes

Spice	Sesquiterpene studied	Spice	Sesquiterpene studied
Citrus sinensis	(+)-Valence	***Polygonum hydropiper***	Polygodial
Citrus aurantifolia	β-Caryophyllene *Trans*-α-bergamotene	***Myrtus communis***	β-Elemene α-Humulene
***Illicium* spp.**	Anethole Anisatin	***Salvia officinalis***	β-Caryophyllene α-Humulene
Cinnamomum zeylanicum	β-Caryophyllene α-Copaene	***Satureja avromanica***	Spathulenol β-Bourbonene
Ocimum sanctum	β-Caryophyllene α-Bulnesene β-Elemene α-Humulene	***Mentha* spp.**	Cubenol β-Caryophyllene Isoledene (-)-Calamene
Matricaria recutita	Matricarin Achillin Acetoxyachillin Leucodin	***Warburgia* spp.**	Polygodial Muzigadial Warburganal Ugandensidial
Curcuma aromatica	(4*S*,5*S*)-(+)-Germacrone-4,5-epoxide	***Alpinia guinanensis***	Carotol
Artemisia umbelliformis	Hydroperoxieudesmanolides Umbellifolide Costunolide Artemorin Santamarine Reynosin	***Laurus nobilis***	δ-Elemene β-Elemene Germacrene D β-Caryophyllene α-Bisabolene
Curcuma zedoaria	Isofuranodienone Glechomanolide Curcumenol Isocurcumenol Procurcumenol Curdione Neocurdione	***Zingiber zerumbet***	Zerumbone

A more detailed study on sesquiterpenes in spices showed that there are more than those listed in Table 4. Nevertheless, it is important to discuss whether the compounds will be absorbed after consumption. The guaianolides matricarin, achillin, acetoxyachillin, and leucodin, which are found in chamomile herb, are only identified in dichloromethane extracts (Tschiggerl and Bucar 2012). In some cases, products are sold after their sesquiterpene properties have been indicated, but this is certainly not always the case. The terpenoids can act in the organism if they are employed in cooking with water. It is therefore important to be cautious about properties described by food developers and when regarding food consumption.

According to Table 4, β-caryophyllene is the main component in spices. In addition, this compound has shown impressive bioactivities against different illness and also as a preventive drug. Degradation of β-caryophyllene, as well as its side effects, was studied, but the results did not show evidence of toxicity to humans. As a consequence, a high-caryophyllene diet should be promoted by the international community.

15.9 Patents

15.9.1 Patents Involving Trichothecenes

There are numerous patents concerning the detection or detoxification process for trichothecenes. This is the case for the combination method involving yeast and different *Bacillus* species as a low cost and environmentally friendly method to detoxify DON. An absorbent composed of a zeolite has been shown to bind specifically with DON, and it is proposed as a method to remove DON from animal feed.

In another case, an enzyme complex containing amidase and esterase is used to remove a group of mycotoxins simultaneously from cereal-based products. The complex is able to remove fumonisins, ochratoxin A, and T-2 toxin efficiently and DON, aflatoxin, and zearalenone partially.

A method has been described to construct a recombinant vector expressing three genes (ADH, AKR6D1, and AKR13B2) involved in the production of proteins to transform DON into a less toxic derivative. It has been proven *in vitro* that these three proteins can jointly transform DON into 3-isomer-deoxynivalenol (3-epi-DON) (Fig. 2), which is much less toxic than DON itself. The first step starts with oxidation, and this transforms DON into 3-oxo-DON, which is subsequently reduced to 3-*epi*-DON.

Another patent concerns extraction methods for different mycotoxins from cereal and other food. This approach involves different steps that allow the toxins to be extracted based on their hydrophilic or hydrophobic properties. The method is able to extract different kinds of aflatoxins, fumonisins, and trichothecenes (DON and T-2 toxin).

15.9.2 Patents Involving Sesquiterpenes Found in Spices and Herbs

There are very few patents related to sesquiterpenes in herbs. One of the most relevant is related to the production of these compounds by cyanobacteria. The gene sequence has been identified, and different sesquiterpenes, such as artemisinin, valence, and bergamotene, can be synthesized as a function of the substrate used to feed them.

Furthermore, the preparation from *Stereum hirsutum* fungus of a novel antidiabetic sesquiterpene has been patented, and the product decreases blood sugar and fructosamine in mice (CN103141300A 2013).

Other patents related to sesquiterpenes are focused on isolation techniques, the development of new extraction methods, or the recovery of volatile compounds. In the cases of ginger, clove, and garlic, an enzymatic process having cellulase, pectinase, protease, and xylanase has been applied to collect the purified spice oil of the herbs, and this oil is rich in sesquiterpenes. The compound zingiber is the subject of the highest number of patents due to its broad application in Asian countries. Bisabolane-type sesquiterpenes isolated from this plant have been patented for use in the pharmaceutical industry and other manufacturing areas. Specifically, an oleoresin is employed to catch the bisabolane.

Finally, separation methods for enriched oil have been patented. A novel distillation device with a dual function of extraction and separation has been employed to collect low-boiling point and high-boiling point components of essential oils in herbs. This system is characterized by a reflux extractor formed by a condenser, a cooler, a high-level oil-water separator, and a water return pipe connected to each other by steam pipes. Furthermore, a countercurrent chromatography method has been patented to isolate chloranthalactone A from *Chloranthus spicatus* by using a mixture of solvents composed of hexane/ethyl acetate/methanol/water (1–3:5–15:2–7:4–15).

15.10 Perspectives

As stated in this chapter, the main problem associated with trichothecenes is their toxicity and the inability to completely remove them in industrial processes. Many studies have shown that a feasible way to decrease greatly their toxicity is the reduction of the epoxy group. This reduction has been achieved at laboratory scale and in biological systems, but it has not been studied on an industrial scale. The authors believe that it would be of interest to carry out an in-depth study in this matter, as cereals play a fundamental role in diet worldwide and there is a continuous exposure of humans to these toxins.

As mentioned in the section on toxicity, a detailed study of star anise should be carried out to determine the maximum safe quantity that can be consumed. The duality aspect of profitable antibiotic activity and the adverse effects of anisatin have not been studied in detail. Furthermore, other plants employed as culinary herbs – such as laurel, which contains ledol – have similar problems that must be analyzed. In the authors' opinion, future efforts on sesquiterpenes related with food with high

levels of consumption have to be focused on determining just how dangerous these kinds of foodstuffs are.

According to clinical studies, it is clear that the potential use of sesquiterpenes as nutraceutical compounds in food has been poorly studied. The last stage of a bioassay in chemistry is difficult, but the sesquiterpenoids analyzed in herbs and cereals are abundant, and they have contrasting benefits in humans. As a consequence, clinical studies related to different illnesses should be performed and not just preventive uses or laboratory tests.

15.11 Cross-References

- ▶ Antioxidants in Diets and Food
- ▶ Curcumin in Food
- ▶ Sesquiterpenes in Fresh Food

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Dietary Monoterpenoids

16

Yoshinori Asakawa

Contents

Abstract

A great number of foods, vegetables, fruits, and beverages contain various types of monoterpenoids and their related compounds. This chapter summarizes the distribution of monoterpenoids in dietary foods, vegetables, fruits, beverages, and processed foods, and their biological activity, effects, and application to our health, as well as metabolic pathways of several monoterpenoids in mammals.

Keywords

Monoterpenoids · Flavor · Essential oils · Biological activity · Biotransformation · Benefits

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16.1 Introduction

Since human being's ancestors began bipedal locomotion, they understood which foods are safe, poisonous, or toxic, and which foods are important to keep their health, and obtained their knowledges of conservation methods of foods, to make processed foods, like cheese, butter, as well as beverages, such as red and white wine, and beer, and succeeded in the production of essential oils including many kinds of monoterpenoids which have been used not only in food preservation but also as some drugs and food additives and in aromatherapy. Now we have many kinds of different foods and processed foods, sports and alcoholic, and non-alcoholic drinks and their supplements in food courts, supermarkets, and department stores. Almost all of edible fruits, mushrooms, sea algae, bryophytes, lichens, pteridophytes, and vegetables and processed foods contain secondary metabolites; mono-, sesqui-, di-, tri-, and tetraterpernoids (carotenoids); phytosterols; pigment components (anthocyanins, flavonoids, stilbenoids); vitamins; hormones; etc. We unconsciously take those foods as breakfast, lunch, and dinner with beverages including green, Oolong, and many kinds of herbal tea as well as alcoholic beverage.

In this chapter, the distribution of monoterpenoids and related compounds in the representative foods, fruits, vegetables, and beverages and their biological activity and metabolism in mammalian body and application are summarized.

16.2 Distribution of Monoterpenoids and Related Compounds in Foods, Fruits, Vegetables, and Beverages

In Table 1, a number of different kinds of foods, fruits, vegetables, and beverages have alphabetically been listed, and the distribution of monoterpenoids and their related compounds as well as their biological effects and application have also been briefly described (Awano et al. 2002; El-Zaeddi et al. 2016; Gottardi et al. 2016; Lasekan and Azeez 2014; Maarse 1991; Kotan et al. 2007; Umemoto et al. 1994; Williams 2011; http://botanicallove.com; https://essentialoils.co.za/essential-oils/citronella.htm; https://www.healthline.com/health/essential-oils-find-the-right-one-for-you#12; http://www.tipdisease.com/2014/11/benefits-and-nutrition-of-chives-allium.html; http://tropical.theferns.info/viewtropical.php; https://www.economie.gouv.fr/files/files/directions_services/dgccrf/imgs/breve/2014/documents/harmonized_list_Section_A.pdf; https://www.5amtag.ch/en/wissen-en/phytochemicals/).

Each monoterpene is listed according to the retention times appeared in gas chromatography (GC) and/or GC-mass spectrometry (GC/MS).

Monoterpenoids are composed of two isoprene units and therefore contain ten carbon atoms (C10), except for the presence nor- (C9) (**67-69**, **131**, and **301**), homomonoterpene (C11) (**74**, **111**, and **242**), and dihomo-monoterpenoids (C12) (**93** and **115**) which are rare natural products. Up to now, over 1,500 monoterpenoids are

Table 1 The dietary plant and fungi used as foods, beverages, and herbal medicines including monoterpenoids and their related compounds and application, benefits, and effects

Abelmoschus esculentus	β-Cyclocitral (**73**)	Vegetable, beverages (herbal tea) Anticancer, antidiabetes, antioxidant For constipation, leucorrhea, spermatorrhea, jaundice, gastritis, gastric ulcers
Averrhoa carambola (Star fruit)	Cumene (**68**), α-terpineol (**312**), carvomenthone (**41**), geraniol (**97**), β-damascenone (**84**), β-ionol (**116**), 4-hydroxy-β-ionone (**123**)	Fruit, beverages Antidiarrheal, antiinflammatory, antioxidant For digestion and immune system booster, promoting healthy hair, nails, and skin
Abies firma	α-Pinene, camphene, Δ^3-carene, bornyl acetate	Beverages (herbal tea) Antidiabetes, antifungal, antimicrobial
Abies sachalinensis var. *mayriana* (Abies oil)	Santene (**301**), tricyclene (**336**), α-pinene (**254**), camphene (**23**), (*Z*)-sabinene (**294**), β-pinene (**255**), myrcene (**191**), Δ^3-carene (**31**), limonene (**152**), *p*-cymene (**79**), β-phellandrene (**255**), α-phellandrene (**254**), terpinolene, (**316**), linalool (**158**), fenchyl acetate (**92a**), pinocarveol (**258a**), bornyl acetate (**15**)	Foods, flavor source (soap, bath additive, deodorant) Antifungal, antioxidant
Acacia decurrens var. *dealbata* (Mimosa oil)	α-Pinene, β-pinene, β-sabinene (**295**), α-phellandrene, myrcene, α-terpinene (**308**), γ-terpinene (**309**), terpinolene (**316**), α-fenchyl alcohol (**89**), linalool, terpinen-4-ol (**307a**), α-terpineol, nerol (**234b**), geraniol, geranyl acetate (**99**)	High-grade compound, flagrance, perfume retention agent Antidepressant, antistress, hypersensitivity, uplifting For anxiety, depressed mood, emetic, skin nourishment, urinary tract infection
Acacia farnesiana (Cassie oil)	*p*-Menth-1-ene (**221a**), limonene, (*E*)-β-ocimene, linalool, camphor (**29**), β-cyclocitral (**73**), nerol, geraniol, α-terpineol, geranial (**94**), thymol (**333**), methyl geranate (**96**), citronellyl acetate (**59**), neryl acetate (**236**), geranyl acetate, (*E*)-α-ionone, dihydro-β-ionone (**125**), geranyl acetone (**111**), β-ionone (**122**)	High-grade fragrance, food flavor (for raspberry) Antipyretic, antispasmodic, aphrodisiac, stimulant For conjunctivitis, dysentery, skin inflammation, tuberculosis

(continued)

Table 1 (continued)

Achillea millefolium (Yarrow oil, Milfoil oil)	Tricyclene, α-thujene (**329**), α-pinene, camphene, β-sabinene, β-pinene, myrcene, α-terpinene, *p*-cymene, limonene, 1,8-cineol (**52**), camphor, γ-terpinene, terpinolene, chrysanthenone (**50**), (*E*)-pinocarveol (**258a**), borneol, terpinen-4-ol, α-terpineol, (*Z*)-piperitol (**263**), carvotanacetone (**46**), piperitone (**81**), (*Z*)-chrysanthenyl acetate (**49b**), bornyl acetate	Beverages Antibacterial, antidepressant, antiinfectious, antifungal, antiinflammatory, antimicrobial, antioxidant, antiphlogistic, antirheumatic, antisclerotic, antiseptic, antispasmodic, astringent, balsamic, calmative cicatrizing circulatory, cytophylactic decongestant, deodorant, digestive, disinfectant, diuretic, emollient, expectorant, febrifuge, hemostatic, hypotensive, immunostimulant, laxative, regenerative, restorative, spasmolytic, stimulant, stomachic, thermogenic, tonic, vasodilatory, vulnerary For emmenagogue
Acorus calamus (Calamus oil)	α-Pinene, camphene, sabinene, myrcene, Δ^3-carene, *p*-cymene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene, linalool, camphor, (*Z*)-β-terpineol (**310**), borneol, terpinene-4-ol (**307a**), α-terpineol (*E*)-carveol, citronellol (**58**), geraniol, methyl citronellate (**57**), bornyl acetate	Foods, beverage industry (for beer, liquor, tonics, tooth powders) Anesthetic, antibacterial, anticancer, anticholinesterase, anticonvulsive, antidiabetes, antidiarrheal, antifungal, antigastrointestinal, antihyperlipidemic, antiinflammatory, antioxidant, antiproliferative, antispasmodic antitoxic, myoelectric action, immunomodulatory For appetite, colic, earache, headache, hemorrhoids, memory problems, neuralgia, poor circulation, rheumatoid arthritis, strokes, skin disorders, ulcers, upset stomach
Acorus gramineus	α-Pinene, camphene, β-pinene, limonene, 1,8-cineole (**52**), *p*-cymene, linalool, camphor, borneol	Foods, beverages Antibacterial, antifungal, antimicrobial, antiperiodic, antirheumatic, antispasmodic, diuretic, sedative, stimulant, stomachic, tonic, vermifuge For anodyne, cardiac carminative, diaphoretic, depression, digestive, emmenagogue, epilepsy, expectorant, febrifuge, sudorific

(continued)

Table 1 (continued)

Actinidia arguta (= *A. japonica*)	α-Pinene, β-pinene, sabinene, myrcene, (*Z*)-β-ocimene, (*E*)-β-ocimene, limonene, 1,8-cineole, terpinolene, linalool, camphor, (7*E*)-8-hydroxylinalool (**159a**), geranyl acetone, (7*Z*)-8-hydroxyllinalool (**159b**), lilac alcohol a-d (**142-145**), lilac alcohol formate 1 (**151**), lilac alcohol formate 2 (**151**), lilac aldehyde 1-4 (**146-149**), (*E*)-linalool oxide (**164a**), (*Z*)-linalool oxide (**164b**), β-ionone	Fruit, beverages Anticancer, antifatigue, antihypercholesterolemia, antiinflammatory, antioxidant, tonic, felis attractant For digestion
Actinidia kiwi	*Fruits*: α-pinene, β-pinene, Δ^3-carene, myrcene, limonene, 1,8-cineole, (*Z*)-β-ocimene, (*E*)-β-ocimene, *p*-cymene, linalool, α-terpineol, geraniol, carvone (**42**), geranyl acetone *Fruits: by acid and enzymatic hydrolysis*: 1,4-Cineole (**51**), 1,8-cineole, *p*-cymene, isophorone (**131**), (*E*)-linalool oxide, (*Z*)-linalool oxide, terpinen-1-ol (**306**), carvenone (**36**), phellandral (**265**), geraniol, *p*-cymenol (**83**), cumin alcohol (**70**), 3-oxo-β-ionone (**118**)	Foods, beverages Antiallergenic, anticancer, antidiabetes, antihypercholesterolemia, antihypertension, antiinflammatory, antioxidant For bone, tooth, vision strengthen, brain development, infant growth, immunity booster, skin health
Actinidia polygama	Actinidin (**1**), iridomyrmecin (**128**), matatabilactone (**18**), pulegone (**267**), *neo*-matatabiol (**175b**), 5-hydroxymatatabidiether (**176**), 7-hydroxymatatabi-diether (**177**), actinidiolide (**2**), actinidol (**4**), *allo*-matatabiol (**175a**), dehydroiridodial (**127b**), dihydroactinidiolide (**3**), dehydroiridodiol (**127a**), *iso*-dihydronepetalactone (**227**), *iso*-iridomyrmecin (**129**), matatabi ether (**179**), matatabiether (**178**), *neo*-nepetalactone (**225**), nepetalic acid (**230**)	Beverages, medicinal liquor Antiseptic, CNS-paralytic, insecticide, pesticide, proteolytic

(continued)

Table 1 (continued)

Aesculus hippocastanum	1,8-Cineole, (*Z*)-linalool oxide, (*E*)-linalool oxide	Tonic as foods Anesthesia, antiinflammatory, antioxidant, antipyretic, astringent, promoting blood circulation For arthritis, cough, dysentery, diarrhea, enlarged prostate, fever, hemorrhoids, menstrual pain, phlebitis, soft tissue swelling from bone fracture, sprains, skin lupus, skin ulcers, varicose veins
Agaricus bisporus	α-Terpineol	Vegetable Anticancer, anticholesterolemic, antidiabetes, antihypoglycemic, antihypolipidemic, antiinflammatory, antimicrobial, antinociceptive, antioxidant, immunomodulatory
Agastache rugosa (Korean mint) (Agastache oil)	α-Pinene, myrcene, limonene, menthone (**189**), pulegone, *iso*-pulegone (**268**)	Flavoring (for salad), beverages Antibacterial, anticancer, antifungal, aromatic, carminative, diaphoretic, febrifuge, stomachic For abdominal bloating, angina pain, chest congestion, cold symptom, diarrhea, dietary indiscretion, headaches, nausea, vomiting
Akebia quinata	Limonene, *p*-cymene terpinolene, linalool, borneol, (*Z*)-sabinene hydrate (**297**), terpnen-4-ol, bornyl acetate, (*E*)-carveol	Foods For stomach and menstrual pain, cystitis, inflammation, stomachache, ulcer, uretic
Allium chinense (Baker's garlic)	Linalool	Vegetable (garnish on salads) Astringent, carminative, expectorant For stuffiness sensation, chest pain, angina pectoris, pleurisy, bronchitis, diarrhea, tenesmus in cases of dysentery
Allium schoenoprasum (Chive)	Linalool, thymol	Vegetable Antibacterial, anticancer, antifungal, antiinflammatory, antihypertension For acne, Alzheimer's disease, arthritis, eye sight, insect repellent, digestion, rheumatoid

(continued)

Table 1 (continued)

Allium tuberosum (Chinese chives)	*Fresh leaves:* α-terpineol, nerol, geraniol *Stoked 30 days*: β-pinene, linalool	Vegetable Anticancer, antiinflammatory, antimicrobial, antioxidant For anemia, atherosclerosis, coronary heart disease, bone booster, digestion, detoxification immunity, skin, vision health
Aloe arborescens	Limonene, carvone (**42**),	Foods, beverages Analgesic, antibiotic, antifungal, antimicrobial, antiinflammatory, antioxidant, antiseptic, antitumor, antiviral, cicatrizing, cytotoxic, depurative, diuretic, eupeptic, gastroprotective, laxative, toning
Aloe ferox	α-Pinene, camphene, β-pinene, Δ^3-carene, limonene, 1,8-cineole, α-terpinene, α-terpinolene, *o*-cymene (**77**), bornylene (**22**), theaspirane (**319**), bornyl acetate	Foods, beverages Antiinflammatory, hypoglycemic, laxative For asthma, dermatitis, diabetes, dry skin, genital herpes, hepatitis, immune system booster, psoriasis, seborrheic, stomachache, ulcer, sunburns
Alpinia japonica (Alpinia oil)	Tricyclene, α-pinene, camphene, α-phellandrene, limonene, α-fenchene (**88**), (*E*)-β-ocimene, *p*-cymene, 1,8-cineol, fenchone (**92a**), camphor, borneol, myrtenol (**196a**), terpinene-4-ol, pinocarvone (**260**), α-terpineol, α-fenchyl alcohol, β-fenchyl alcohol (**91**), β-fenchyl acetate	Beverages Antiinflammatory, antitumor For diarrhea, hematemesis, menoxenia, muscle pain, stomachache
Alpinia speciosa	*Leaves:* tricyclene, α-thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, limonene, γ-terpinene, 1,8-cineole, (*Z*)-β-ocimene, (*Z*)-sabinene hydrate, terpinolene, linalool, camphor, (*E*)-*p*-menth-2-en-1-ol (**211a**), (*Z*)-*p*-menth-2-en-1-ol (**211b**), terpinen-4-ol, α-terpineol, β-terpineol (**310**), borneol, (*E*)-piperitol (**262**), myrtenal, (*Z*)-carveol, (*E*)-carveol, cumin aldehyde, α-	Foods, food additives (cookies, sponge cakes, noodles), beverages Antiaging antiallergenic, antibacterial, antifungal, antihypertension, antiinflammatory, antimicrobial, antioxidative, diuretic, spasmolytic, insecticide, sedative For cardiovascular disease, menstrual cramps, muscle pain

(continued)

Table 1 (continued)

	phellandral (**265**), thymol, linalyl *iso*-valerate (**172**), terpinyl pentanoate (**314b**) *Flowers:*α-thujene (**329**), α-pinene, camphene, sabinene, myrcene, α-phellandrene, α-terpinene, 1,8-cineole, (*E*)-β-ocimene, γ-terpinene, (*Z*)-sabinene hydrate, terpinolene, linalool, (*Z*)-*p*-menth-2-en-1-ol, (*E*)-*p*-menth-2-en-1-ol, camphor, terpinen-4-ol, α-terpineol, (*E*)-piperitol (**262**), bornyl acetate *Seeds:* α-pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, terpinolene, linalool, fenchyl alcohol, (*E*)-*p*-menth-2-en-1-ol, camphor, β-terpineol, pinocarvone (**260**), pinocamphone (**261**), terpinen-4-ol, α-terpineol, borneol, (*E*)-piperitol (**262**), myrtenal, (*Z*)-carveol, (*E*)-carveol, cumin aldehyde, thymol, geranyl acetate, linalyl *iso*-valerate (**172**) *Whole plant*: fenchene, (*E*)-pinocarveol (**258a**), *iso*-borneol (**19a**), ascaridole (**9**), nerol, myrtenol, geraniol, fenchyl alcohol, *p*-cymene-8-ol (**80**), thymol, carvacrol, (*E*)-*p*-mentha-1(7),8-dien-2-ol (**203a**)	
Alpinia zerumbet	α-Thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, α-terpinene, limonene, β-phellandrene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, *p*-cymene, terpinolene, linalool, (*Z*)-pinocarveol (**258b**), camphor, terpinene-4-ol, dill ether (**86**), α-terpineol, carvone, *p*-menth-1-en-9-ol (**206**)	Beverages Anticancer, antidiabetes, antihypertension, antiinflammatory, antimalarial, antimicrobial, antinociceptive, antioxidant, antipyretic, antispasmodic, antiulcerogenic, diuretic, neuropsychiatric syndrome For cardiovascular disorders, cold
Amanita rubescens	α-Pinene, β-pinene, limonene, 1,8-cineole, linalool, menthol	Foods

(continued)

Table 1 (continued)

Amygdalus persica (= *Prunus persica*)	Sabinene, limonene, *p*-cymene, linalool, linalyl formate (**167**), linalyl acetate (**168**), linalyl pentanoate (**171**), linalyl hexanoate (**174**), β-ionone	Fruit Antiaging, anticancer For healthy bones and teeth, strengthening the immune system, preventing nervous system, cancer prevention, healthy brain, blood pressure control
Amygdalus dulcis (Almond)	α-Thujene, α-pinene, camphene, sabinene, myrcene, limonene, 1,8-cineol, linalool, borneol, *p*-cymene-9-ol, (*E*)-verbenol (**342a**), myrtenol, α-terpineol, carvacrol	Foods Antiaging, antiarteriosclerosis, anticancer, antioxidant, hypocholesterolemia
Ananas comosus	α-Pinene, β-pinene, myrcene, limonene, (*E*)-β-ocimene, (*Z*)-β-ocimene, linalool, terpinen-4-ol, α-terpineol, geraniol	Fruit, beverages Anticancer, antiinflammatory, antioxidant, antiscorbutic diuretic For arthritis, diaphoretic, digestion, gastric irritability, jaundice, laxative
Angelica archangelica	α-Pinene, camphene, β-pinene, sabinene, myrcene, Δ^3-carene, limonene, β-phellandrene, α-phellandrene, *p*-cymene, (*E*)-β-ocimene, (*Z*)-β-ocimene, terpinolene, cryptone (**67**), carvone	Beverages, flavoring agent Antibacterial, anticancer, antifungal, antiinflammatory For acne control, appetizer, blood circulation, digestion, flatulence, hepatoprotective, improving recovery, menstrual and respiratory disorders, rheumatism, relaxant, mouth and skin health, ureter infections
Angelica keiskei	α-Thujene, tricyclene, sabinene, Δ^3-carene, myrcene, β-phellandrene, γ-terpinene, *p*-cymene, α-terpinolene, linalool, *p*-cymen-9-ol, terpinen-4-ol, bornyl acetate, (*E*)-β-ionone, citronellyl butanoate (**61**)	Beverages Antiaging, antibacterial, anticancer, antifungal antihypertension antiinflammatory, antimicrobial, antioxidant, antiparasitic, tonic For anemia, constipation, detoxification, diabetes, gastrointestinal disorder, joint and muscular pain, nerve degeneration, premenstrual syndrome, immune system booster, weight loss

(continued)

Table 1 (continued)

Angelica sylvestris	α-Pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, *p*-cymene, limonene, terpinolene, cryptone, carvone, bornyl acetate	Candies, beverages (herbal tea) Antifungal, antimicrobial, antiparasitic, diuretic, expectorant For anorexia, anxiety, arthritis, circulation problems, cold, coughs, cystitis, dementia, dyspepsia, fever, headaches, indigestion, insomnia, muscular cramps, nervousness and plague, nocturia, respiratory catarrh, rheumatic pain, runny nose, stroke, sore throat flatulence, urinary antiseptic
Aniba rosaeodora (Bois de Rose oil)	Limonene, 1,8-cineole, linalool, α-terpineol, nerol, geraniol, linalyl acetate	Flavoring, perfumes, cosmetics Analgesic, antibiotic, antiseizure, antiseptic, aphrodisiac, cephalic, deodorant, insecticide For protecting the health of skin, relieving stress and pain, sexual health, sore muscle, source of linalool, spiritual and mediation
Anthemis nobilis (Roman chamomile oil)	α-Pinene, sabinene, myrcene, *p*-cymene, 1,8-cineole, limonene, (*E*)-β-ocimene, artemisia ketone (**5**), α-terpinene, terpinolene, terpinen-4-ol, α-terpineol, carvone	Beverages, deodorant for liquor, flavor reinforcing agent Anticancer, antiinflammatory, antimicrobial, sedative
Anthriscus cerefolium (Chervil)	β-Pinene	Vegetables, beverages (herbal tea) Antifungal, antihypertension, antiinflammatory, antioxidant, diuretic, mild stimulant, negative inotropic, spasmolytic For digestion
Anthriscus cerefolium (Wild chervil)	α-Pinene, sabinene, myrcene, β-phellandrene, (*Z*)-β-ocimene	Vegetables, beverages (herbal tea) Analgesic, antiinflammatory, antiplatelet aggregation, antiproliferative, antipyretic, antitussive, antitumor, antiviral, diuretic For cough remedy

(continued)

Table 1 (continued)

Apium graveolens var. *dulce* (Celery)	α-Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, cumene (**68**), α-terpinene, limonene, β-phellandrene, *p*-cymene, γ-terpinene, (*E*)-β-ocimene, terpinolene, *p*-mentha-1,3,8-triene (**204**), borneol, terpinene-4-ol, carveol	Vegetable (salad, cooked vegetable, in soup, stews) Antibacterial, anticancer, antihypertension, antiinflammatory, antimicrobial, antioxidant, antiulcerogenic For bladder and liver disorders, stomach digestion, urinary tract infections
Apium graveolens var. *dulce* (Celery)	*Seed oil*: α-pinene, β-pinene, myrcene, limonene, (*E*)-β-ocimene, γ-terpinene, *p*-cymene, linalool, (*E*)-limonene 1,2-oxide (**154a**), dihydrocarvone (**43**), *p*-mentha-6,8-dien-2-ol, α-terpineol, (*E*)-carveol, carvone, (*E*)-sabinyl acetate (**299**), (*Z*)-carveyl acetate (**39b**), (*Z*)-*p*-mentha-2,8-dien-1-ol (**209b**) *Leaves*: α-pinene, β-pinene, myrcene, limonene, γ-terpinene, *p*-cymene, (*E*)-limonene-1,2-oxide (**154a**), (*Z*)-limonene-1,2-oxide (**154b**), α-terpineol, dihydrocarveol (**37**), (*E*)-*p*-mentha-2,8-dien-1-ol (**209a**), (*Z*)-p-metha-2,8-dien-1-ol (**209b**), (*Z*)-*p*-mentha-1(7),8-diene-2-ol (**203b**), (*E*)-*p*-mentha-1(7),8-dien-2-ol (**203a**), (*Z*)-carveol (**39a**), (*E*)-carveol (**38a**), *p*-mentha-8-en-1,2-diol, dihydrocarvone, carvone, (*Z*)-*p*-mentha-1(7),8-dien-2-yl acetate (**203c**), (*E*)-carveyl acetate (**38b**), pinocarveyl acetate (**259**)	Vegetable Analgesic, antiarteriosclerosis, anticancer, antihypertension, analgesic, diuretic, platelet condensation For constipation, whole intestine
Apium graveolens var. *rapaceum*	α-Pinene, sabinene, myrcene, α-terpinene, limonene, β-phellandrene, γ-terpinene, (*E*)-β-ocimene, *p*-cymene, linalool, terpinen-4-ol, α-terpineol	Vegetable Antiosteoporosis, antipneumonia, antitumor, diuretic For brain and muscle function, digestion, gout, immunity system booster, digestion, Parkinson's disease, brain and muscle, function, rheumatism, skin health, wounds

(continued)

Table 1 (continued)

Arachis hypogaea	Myrcene, limonene, β-terpinenyl acetate (**311**)	Foods Antiaging, anti-Alzheimer disease's, antidiabetes, antihypertension, antiinfluenza, antioxidant
Aralia cachemirica	α-Pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, 1,8-cineolol, (*Z*)-β-ocimene, linalool	Anticancer, antibacterial, antiinflammatory For arthritis, diabetes mellitus, gastritis, hypoglycemia, nephritis, rheumatic lumbago, lameness
Aralia cordata	α-Pinene, camphene, β-pinene, sabinene, Δ^3-carene, myrcene, α-phellandrene, limonene, terpinolene, (*Z*)-*allo*-ocimene (**245**), (*E*)-*allo*-ocimene (**246**), *p*-cymene, camphor, borneol, *p*-cymen-8-ol, terpinen-4-ol, carvone, α-terpineol, (*E*)-sabinene hydrate (**296**), (*E*)-carveol, β-citronellol, (*E*)-pinocamphone (**261**), α-thujone (**331a**), verbenone, (*E*)-pinocarveol, lavandulol (**136**), *p*-methadien-2-ol, thymol	Vegetable Antipyretic, diaphoresis, sedative For cold, sensitive constitution
Aralia elata (Angelica tree)	α-Pinene, camphene, β-pinene, (*E*)-β-sabinene (**294**), myrcene, α-phellandrene, α-terpinene, limonene, β-phellandrene, (*Z*)-β-ocimene, γ-terpinene, (*E*)-β-ocimene, *p*-cymene, α-terpinolene, (*Z*)-linalool oxide, (*E*)-linalool oxide, myrtenol, linalool, camphor, borneol, myrtenal (**195**), *p*-cymen-9-ol, terpinen-4-ol, α-terpineol, nerol (**234b**), geraniol, geranial, bornyl acetate, geranyl formate (**98**), geranyl butanoate (**101**), geranyl hexanoate (**107**), geranyl octanoate (**109**), geranyl benzoate (**110**), neryl *iso*-valerate (**237b**), neryl hexanoate (**239**), bornyl hexanoate (**18**), citronellyl hexanoate (**65**), citronellic acid (**56**), geranic acid (**95**), β-ionone, (*Z*)-jasmone (**133**)	Vegetable Antidiabetes, antimicrobial, antioxidant, sterilization For blood improvement, immune system booster

(continued)

Table 1 (continued)

Arctium lappa	Neral, geranial, geraniol	Beverages (detoxifying herb) Antibacterial, anticancer, antifungal, antihepatotoxic, antiinflammatory, antimicrobial, antimutagenic, antioxidant, antitumor, antiulcer, diaphoretic, diuretic, hypoglycemic For carminative, cholagogue, depurative, eczema, ringworm, stomachic, skin disease
Arctostaphylos uva-ursi	α-Thujene, β-thujene (**330**), limonene, *p*-cymene, linalool, camphor, (*Z*)-linalool oxide, (*E*)-linalool oxide, borneol, *p*-cymene-9-ol, myrcenol (**192**), α-campholenal (**28**), (*E*)-pinocarveol, lilac aldehyde B (**147**), lilac aldehyde A (**146**), nerol oxide (**242**), (*Z*)-β-ocimenol (**247**), menthol (**183**), lilac aldehyde C (**148**), α-phellandren-8-ol, (**270**), *p*-mentha-1,5-dien-8-ol,(**217**), *iso*-menthol (**186a**), terpinen-4-ol, *iso*-verbanol (**340a**), *neo*-isomenthol (**188**), α-terpineol, myrtenol, γ-terpineol, verbenone, (*E*)-carveol, *p*-menth-1-en-9-al (**221d**), perilla alcohol (**271**), nerol, geraniol, β-cyclocitral, carvone, perilla ketone, (**273**), pulegone, carvacrol methyl ether (**35**), piperitone, (**281**), perillaldehyde (**274**), *iso*-menthyl acetate (**186b**), thymol methyl ether (**334**), thymol, carvacrol, (*E*)-β-damascenone (**85**), (*Z*)-jasmone (**133**), (*E*)-geranyl acetone, dihydroactinidiolide, (*Z*)-β-ocimenone (**249**)	Foods For bladder and kidney disorders, bronchitis, constipation, inflammatory diseases of the urinary tract, urethritis, cystitis, strengthening and imparting tone to the urinary passages, swelling of the bladder, urethra, constipation, urethritis

(continued)

Table 1 (continued)

Artemisia absinthium (Worm wood)	α-Thujene, α-pinene, verbenene, sabinene, β-pinene, myrcene, α-phellandrene, α-terpinene, (*E*)-sabinene hydrate, linalyl acetate, camphor, (*E*)-verbenol, thujone, α-campholenal, sabinaketone, pinocarvone, umbellulone, terpinen-4-ol, myrtenal, verbenone, β-terpinyl acetate (**311**), (*Z*)-chrysanthenyl acetate (**49**), neryl isobutanoate (**237a**), geranyl isobutanoate (**102**), neryl isovalerate (**237b**)	Beverages (herbal tea) Anthelmintic, antioxidant, antiseptic, cardials, insecticidal, narcotic, diaphoretic For Crohn's disease, loss of appetite, indigestion, gallbladder disorders, low sexual desire, spasms, sweating stimulant, worm infestations, wounds
Artemisia annua	α-Pinene, camphene *p*-cymene, camphor, 1,8-cineole, 3(10)-caren-4-ol (**32**), α-terpineol, carvone, artemisia ketone (**5**), artemisyl acetate (**7**), (*Z*)-chrysanthenyl acetate (**49**), yomogi alcohol (**345**)	Beverages Anthelminthic, anticancer, antimalarial, antipyretic, antiseptic, antispasmodic, antiviral For carminative, cold, cough, diarrhea, nervous diseases, stimulant, tonic, stomachic
Artemisia capillaris	α-Pinene, β-pinene, Δ^3-carene, limonene, γ-terpinene, *p*-cymene, α-terpineol, bornyl acetate	Beverages Antifibrotic, antiinflammatory, antioxidant, antisteatotic, antiviral, cholagogue, choleretic, vermicide
Artemisia dracunculus (Tarragon oil or estragon oil)	α-Pinene, camphene, sabinene, β-pinene, myrcene, *p*-cymene, limonene, α-phellandrene, (*Z*)-β-ocimene, (*E*)-β-ocimene, *allo*-ocimene (**246**), γ-terpinene, α-terpinene, (*Z*)-sabinene hydrate (**297**), linalool, β-thujone (**331b**), camphor, terpinen-4-ol, *iso*-menthol (**186a**), bornyl acetate, carvacrol, α-terpinyl acetate (**313**)	Flavoring and fragrance, beverages, source, vine flavor Analgesic, antidiabetes, antiinflammatory, antimicrobial, sedative For osteoarthritis, sleep aid
Artemisia indica var. *maximowiczii*	α-Pinene, camphene, sabinene, myrcene, limonene, 1,8-cineol, *p*-cymene, camphor, (*E*)-sabinene hydrate, verbenol, borneol, chrysanthenone, (*E*)-chrysanthenyl acetate, (**48a**), verbenol, 2-pinen-4-ol [(=(Z)-verbenol)] (**343**), terpinen-4-ol, thujone, *p*-menth-1-en-8-ol (**205**), bornyl acetate, lavandulyl acetate (**137**)	Foods, food additives, food flavor for Japanese cookies, Mochi (sticky rice boll) Antipyretic For abdominal pain, anemia, back pain, diarrhea, hemostatic, poor circulation, stomachic

(continued)

Table 1 (continued)

Artemisia indicium var. *indicum*	α-Pinene, myrcene, limonene, 1,8-cienole, (*Z*)-β-ocimene, chrysanthenone (**50**), camphor, borneol, bornyl acetate	Foods (leaves for Japanese cuisine) Antimicrobial
Artemisia princeps (Japanese mugwort)	α-Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, 1,8-cineole, β-phellandrene, (*Z*)-β-ocimene, γ-terpinene, (*E*)-β-ocimene, *p*-cymene, terpinolene, *o*-cymene, dehydro-*p*-cymene (**75**), 2,3-dehydro-1,8-cineole (**53**), artemisia ketone, yomogi alcohol, terpinen-4-ol, α-thujone, artemisyl acetate (**7**), β-thujone (**331b**), camphor, (*E*)-verbenol, borneol, (*E*)-sabinene hydrate, chrysanthenone, artemisia alcohol (**8**), thujanol acetate (**325b**), (*Z*)-sabinene hydrate, pinocarvone, myrtenal (**195**), *p*-cymen-9-ol, citronellol, (*E*)-myrtenol, (*Z*)-myrtenol, α-terpineol, carvone, thujyl alcohol (**326**), α-terpinyl acetate, carveyl acetate,, verbenone (**344**), (*Z*)-carveol, (*E*)-carveol, neral (**234a**), geranial, geraniol, piperitol, chrysanthenol (**47**), bornyl acetate (**15**), lavandulyl acetate (**137**), bornyl propanoate (**16a**), bornyl isobutyrate (**16b**), bornyl angelate (**16c**), (*Z*)-myrtenol (**194b**), (*E*)-myrtanol (**194a**), perilla alcohol (**271**), perillaldehyde, *p*-mentha-1(7),8-triendien-2-ol, *iso*-piperitenone (**276**), piperityl acetate (**264**)	Foods, beverages Analgesic, anticancer, expectorant, hemostatic For anemia prevention, blood circulation, cerebral infarction prevention, constipation, hematogenesis, menstrual regulation, myocardial
Artemisia tridentata	Artemisia triene (**6b**), santolina triene (**301b**), α-thujene, α-pinene, camphene, 1,8-cineol, chrysanthenone, camphor, pinocarvone, geranyl *iso*-butanoate (**102**), geranyl butanoate (**101**), neryl *iso*-valerate (**237b**), geranyl *iso*-valerate (**104**)	Perfume, beverages (herbal tea) Antibacterial, antirheumatic, antiseptic, digestive, disinfectant, febrifuge, ophthalmic, poultice, sedative For bronchitis pneumonia, bad colds, coughs, sore throats

(continued)

Table 1 (continued)

Asparagus officinalis	Geranyl acetone, β-ionone	Vegetable (raw or pickled, cooked) Anticancer, antidiabetes, antimicrobial, antioxidant, antitussive, diuretic, expectorant, fatigue recovery, porcelain skin, hypolipidemic, vermicide For lung heat, convulsions, mange
Atractylodes japonica	Δ^3-Carene, sabinene, *p*-cymene, verbenol	Herbal medicines. Antiosteoporosis For allergies, bloating, diarrhea, dust mites, dialysis, edema, fluid retention, indigestion, joint pain, rheumatism, stomachache, weight loss
Basella rubra	Limonene, 2-menthene (**181**), pulegone, myrcenol, piperitone (**281**), piperitenone (**275**), β-damascenone	Vegetable, beverages (herbal tea) Androgenic, anticancer, antifungal, antihypoglycemic, antimicrobial, antioxidant, cytotoxic, antibacterial, antiinflammatory, antiulcer, antiviral, central nervous system depressant, hypocholesterolemic, immunomodulatory, nephroprotective, gastroprotective For wound healing
Bassia scoparia (Kochia, Mexican fireweed)	Camphenilone (**26**), damascenone, bornyl angelate (**16c**)	Vegetable Antifungal, antimicrobial, antiphlogistic astringent, cardiotonic, diuretic, restorative For colds, diarrhea, dysentery, dyspepsia, eczema, fevers, hernia, incontinence of urine, intercostal neuralgia, hernia
Beta vulgaris var. *rapa* (Table beet, red beet, yellow beet)	Carvone, γ-irone (**130c**)	Vegetable, beverage (juices) Antioxidant
Betula pendula	*p*-Cymene, (*E*)-linalool oxide, (*Z*)-linalool oxide	Foods, alcoholic beverages, aroma, and flavor Anticholesterolemic, antifungal, antiinflammatory, diuretic, germicides, laxative tonic For eczema, intermittent fevers, psoriasis

(continued)

Table 1 (continued)

Boletopsis leucomelas	α-Pinene, *o*-cymene (**77**), (*E*)-pinocarveol (**258a**), menthol (**183**), terpinen-4-ol, *p*-cymen-8-ol (**81**), cryptone (**67**)	Foods Apoptosis induction
Boletus edulis	α-Pinene, limonene, 1,8-cineole, linalool, menthol, (*E*)-geranyl acetone, β-ionone	Foods
Boswellia carterii (Olibanum oil)	α-Pinene, camphene, α-phellandrene, *p*-cymene, verbenol, verbenone, bornyl esters	Beverages Arthritis, anticancer, antiinflammatory For asthma, Parkinson's disease, rheumatoid arthritis
Brassica campestris	Cumene	Vegetable
Brassica oleracea var. *capitata* (Cabbage)	1,8-Cineole, β-cyclocitral, geranyl acetone	Vegetable
Brassica juncea (Leaf mustard)	Linalool, citronellol, nerol, geraniol	Vegetable Antitumor, anodyne, diuretic For abscesses, aperitif, emetic, cold, lumbago, rheumatism, rubefacient, stomach disorders
Brassica juncea var. *integrifolia* (Mustard vegetable)	Pulegone, lavandulol (**136**)	Vegetable, pungent spice Anticancer, antimicrobial, diuretic, expectorants, stimulant
Brassica napus (= *B. napa* var. *amplexicaulis*)	α-Pinene, β-pinene, sabinene, myrcene, limonene, 1,8-cineole, perillene (**277**), linalool, β-ionone	Vegetable Antigout, antiinflammatory, antiscurvy, diuretic
Brassica oleracea var. *acephala* (Kale)	1,8-Cineole, geranyl acetone	Vegetable, beverages Anticancer, antidiabetes, antioxidant For hot flashes, high cholesterol, heart disease, colitis, constipation, loss of vision, wound healing
Brassica oleracea var. *italica* (Broccoli)	Limonene	Vegetable Antiinflammatory, laxative For glaucoma, pneumonia
Callicarpa americana	β-Pinene, α-terpinene, *p*-cymene limonene, 1,8-cineolle, γ-terpinene, *(Z)*-linalool oxide, terpinolene, linalool, β-fenchyl alcohol, (*Z*)-*p*-ment-2-en-ol, α-campholenal, (*E*)-sabinol, pinocarvone, borneol, α-terpineol, myrtenal, myrtenol	Fruit, beverages (herbal tea) Diuretic For dysentery, colic, fever, malaria, rheumatism, stomachaches

(continued)

Table 1 (continued)

Callicarpa japonica	α-Pinene, β-pinene, linalool	Antibacterial, antiviral, hemostatic, insecticidal For intestinal and uterine bleeding
Camellia sinensis	Myrcene, limonene, linalool, *p*-cymene, (*E*)-linalool oxide (furanoid) (**164a**), (*Z*)-linalool oxide (furanoid) (**164b**), linalool oxide (pyranoid), (**165a,b**), jasmone, terpinen-4-ol, α-terpineol, citral, nerol, geraniol, menthol, geranic acid, neryl acetone (**241**), actinidiolide	Beverages Antiarteriosclerosis, anticancer, antiinflammatory, antimicrobial, antioxidant, antiviral, diuretic, hypoglycemic, suppressing blood sugar rise, hypotensive, hypolipidemic, hypocholesterolemia, skin secretion regulating action
Cananga odorata f. *genuine* (Ylang-ylang oil)	α-Pinene, limonene, linalool, geraniol, geranyl acetate	Foods to avoid with arthritis, luxury compounded fragrance Antiaging, antiinflammatory, antioxidant, anxiety, carminative, insect repellent, NO production inhibition For athlete's foot, arthritis, asthma, colds, cough, depression, diarrhea, fever, headaches, insomnia, irregular menstrual period, malaria, joint, menstrual and muscle pain, nervous fatigue, sinus infections, wound healing
Cananga odorata f. *macrophylla* (Cananga oil)	α-Pinene, limonene, linalool, geranyl acetate	Flavoring agent in gelatins, puddings, and beverages, cosmetics for soap Antiinflammatory, antimicrobial For cold, hypertension, insect bites, menopausal disorders, nervous fatigue, skin care
Canavalia gladiata	*	Vegetables, beverages Antiinflammatory For ache, asthma, atopic dermatitis, dysentery, kidney disease, obesity, stomachache, vomiting

(continued)

Table 1 (continued)

Cannabis sativa	*Seed*: α-thujene, α-pinene, camphene, β-pinene, α-terpinene, sabinene, limonene, 1,8-cineol, γ-terpinene, (*E*)-β-ocimene, fenchene	For amyotrophic lateral sclerosis, cachexia or dramatic weight loss, muscle atrophy, cancer, constipation, glaucoma, HIV/AIDS, Huntington's disease, inflammatory bowel, injury with spasticity, multiple sclerosis, muscle spasms, nausea, neuropathy, Parkinson's disease, spinal cord post-traumatic stress disorder, seizures
Cantharellus cibarius	α-Pinene, limonene, 1,8-cineol, menthol	Foods
Capsicum annuum var. *grossum* (Bell pepper, sweet pepper)	*Green, turning, and red stage*: α-pinene, β-pinene, Δ^3-carene, myrcene limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, *p*-mentha-1,3,8-triene, linalool, fenchyl alcohol	Vegetable Anticancer, antimicrobial, antioxidant, antiseptic For appetite stimulator, cough, counter immunomodulatory, headache, irritant, parasitic infection, rheumatism, sore throat, toothache, wound healing
Capsicum frutescens (= *C. annum*) (Red pepper, chili pepper)	*Oleoresin*: β-pinene, Δ^3-carene, myrcene, limonene, α-phellandrene, 1,8-cineole, linalool, terpinen-4-ol, α-terpineol, thujone, β-cyclocitral, carvone, dihydro-α-ionone (**121**), dihydro-β-ionone (**125**), α-ionone, β-ionone, geranyl acetone, dihydroactinidiolide *Sweet chili*: α-thujene, α-pinene, camphene, β-pinene, sabinene, Δ^3-carene, myrcene, limonene, α-phellandrene, γ-terpinene, *p*-cymene, terpinolene, linalool, terpinen-4-ol *Fresh Jalapenos*: linalool, α-terpineol, β-ionone	Vegetable, flavoring, food preservatives Antimicrobial, antifungal, antihemorrhoidal, antiobesity, antirheumatic, antiseptic, carminative, circulation and alters temperature regulation, diaphoretic, detoxifiers, digestive, gastrointestinal circulation, sialagogue, stomachic For cardiovascular disease, metabolic syndrome
Carica papaya	α-Pinene, β-pinene, Δ^3-carene, myrcene, limonene, (*E*)-β-ocimene, (*Z*)-β-ocimene, *o*-cymene, linalool, (*E*)-linalool oxide, (*Z*)-linalool oxide, verbenone	Foods, flavoring used in candies, jellies, preserves, ice cream, meat tenderizers, chewing gum Antidiabetes, antihypertension, antiinflammatory, antimicrobial, antiparasitic, antiseptic, diuretic, hypocholesterolemic

(continued)

Table 1 (continued)

Carthamus tinctorius	*Flower:* α-phellandrene, α-terpinene, limonene, γ-terpinene, *p*-cymene, 1,8-cineole, camphor, terpinen-4-ol, α-phellandrene oxide (**278**), β-damascenone	Beverages For angina, arteriosclerosis prevention, breast cancer, cataract arteriosclerosis, eye strain, poor blood circulation promotion, uterine fibroids, whole intestine
Carum carvi (Caraway oil)	Limonene, dihydrocarvone (**43**), carvone, carveol, dihydrocarveol (**37**), perilla alcohol, carvacrol	Beverages, canned food flavor, liquor of seasoning, sausage, meat, confectionary flavor, spice Antigriping agent, antiinflammatory, antimicrobial, antioxidant, carminative, pediculicide, mild CNS depressant, immunomodulatory, stomachic
Carum copticum (Ajowan oil)	α-Thujene, α-pinene sabinene, myrcene, α-terpinene, limonene, β-phellandrene, γ-terpinene, *p*-cymene, β-fenchyl alcohol (**92a**), (*Z*)-limonene oxide, terpinolene, terpinen-4-ol, thymol, carvacrol	Foods, spice in curries, cosmetics (lotion, ointment) Abortifacient, abortion potential, amebiasis, antiarthritic, antibacterial, anticarcinogenic, antidyspnea, antilithiasis, antimicrobial, antiparasitic, antiplatelet-aggregatory, antiseptic, antitussive, bronchodilatory, carminative, diuretic, expectorant, genotoxicity, galactagogic For acute pharyngitis, cold, diarrhea, digestive, dysentery, gastrointestinal, inflectional respiratory disorders, indigestion, rheumatic pain
Celosia argentea	α-Pinene, camphene, limonene, *p*-menth-3-en-8-ol, β-cyclocitral, pulegone, carvanacetone, geranyl acetone, (*E*)-β-ionone	Beverages (herbal tea) Astringent, hemostatic, ophthalmic, parasiticide, poultice For bloody stool, dysentery diarrhea, hemorrhoid, uterine bleeding, leucorrhoea, menstrual dysfunction
Centaurea cyanus	β-Thujene, α-terpinene, γ-terpinene, *p*-cymene, α-terpinolene, (*E*)-linalool oxide, *p*-cymene-9-ol, terpinen-4-ol, verbenone	Beverages Astringents, skin conditioning

(continued)

Table 1 (continued)

Centella asiatica	α-Pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, Δ^3-carene, Δ^2-carene, α-terpinene, *p*-cymene, limonene, *p*-menth-3,8-diene (**221b**), 1,8-cineole, (*E*)-*allo*-ocimene (**245a**), γ-terpinene, terpinolene, linalool, *m*-cymen-9-ol, terpinene-4-ol, (Z)-dihydrocarvone, verbenone, (*E*)-carveol, carvone, (*E*)-myrtenol, *p*-cymene-7-ol (=cumin alcohol) (**70**), *p*-cymene-9-ol, α-terpineol, geraniol, myrtenyl acetate	Beverages (herbal tea) Antischistosomiasis For anxiety, cold, flu, diarrhea, fatigue, hepatitis, indigestion, jaundice, sunstroke, tonsillitis, urinary tract infection, wound healing
Chaenomeles japonica	α-Pinene, β-pinene, sabinene, Δ^3-carene, myrcene α-phellandrene, α-terpinene, limonene, γ-terpinene, terpinolene, geraniol, carveol, terpinen-4-ol, citronellal, neral, geranial, perillaldehyde, carvone, linalyl acetate, citronellyl acetate, neryl acetate (**237**), α-terpinyl acetate	Beverages, juice wine, puree, aroma extracts, syrup liquor, carbonated soft drink, marmalades, flavored sweet, ice cream included fruit sugar extracts, nourishing tonic Anticonvulsant, antitussive, expectorant For gastrospasm, muscle ache
Chamaecyparis formosensis (Benihi oil)	α-Pinene, camphene, sabinene, myrcene, limonene, 1,8-cineole, γ-terpinene, camphor, borneol, α-terpineol, myrtenol, myrtenal, bornyl acetate, geranyl acetone, dihydromyrtenol (**199**), myrtenyl acetate (**197**)	Compound flavor Antifungal, antimicrobial, larvicidal (mosquito), nitric oxide production inhibitory
Chamaecyparis obtusa (Hinoki oil)	α-Pinene, camphene, β-pinene, limonene, borneol, α-terpineol, bornyl acetate	Compound flavor, soap flavor, bath additives Bactericidal, insecticide
Chamaecyparis obtusa var. *formosana* (Formosan hinoki oil)	α-Pinene, camphene, β-pinene, sabinene, γ-terpinene, *p*-cymene, linalool, α-terpineol, α-thujaplicin (**327**), β-thujaplicin (=hinokitiol) (**328a**)	Compound flavor, soap flavor, bath additives Bactericidal, insecticide
Chenopodium album	α-Pinene, limonene, *p*-cymene, α-terpinene, ascaridole (**9**), pinane-2-ol (=pinene hydrate) (**286** or **287**), α-terpineol, α-terpinyl acetate	Foods, vegetable, herbal medicines Antioxidant, anthelmintic, antiinflammatory, antifungal, antinociceptive, antipruritic, hypotensive, laxative For hepatic disorders, spleen enlargement, intestinal ulcers

(continued)

Table 1 (continued)

Chenopodium ambrosioides (Mexican tea)	α-Pinene, myrcene, Δ^3-carene, α-terpinene, *p*-cymene, limonene, γ-terpinene, pinocarvone, dihydroascaridole (**10**), ascaridole (**9**), (*Z*)-isoascaridole (**11b**), (*E*)-isoascaridole (**11a**), α-terpinyl acetate, thymol	Beverage (herbal tea), vegetable Analgesic, antifungal, antimicrobial, antiinflammatory
Chrysanthemum coronarium (Garland chrysanthemum)	α-Pinene, camphene, β-pinene, myrcene, *p*-cymene, linalool	Beverages, vegetable Anticancer, antiinflammatory
Chrysanthemum indicum	Tricyclene, α-pinene, α-thujene, camphene, β-pinene, sabinene, myrcene, α-terpinene, limonene, α-phellandrene, (*Z*)-β-ocimene, γ-terpinene, (*E*)-β-ocimene, *p*-cymene, terpinolene, 1,8-cineole, linalool, α-thujone, (*E*)-sabinene hydrate, α-terpinyl acetate, chrysanthenone, camphor, pinocarvone, (*Z*)-chrysanthenyl acetate (**49**), bornyl acetate, terpinen-4-ol, umbellulone (**337**), (*E*)-chrysanthenyl acetate (**48**), (*E*)-piperitol, α-terpineol, borneol, piperitone, carvone, (*E*)-chrysanthenol (**47**), myrtenol, (*E*)-carveol (**38a**), *p*-cymen-9-ol, (*Z*)-carveol (**39a**)	Beverages (herbal tea, alcoholic), food additives Antiphlogistic, antimicrobial, blood tonic, hypocholesterolemic, hypotensive For blood stasis, depurative, detoxifying, dissipating blood stasis, dissipating heat, febrifuge, migraine stomachic, vasodilator, vulnerary
Chrysanthemum morifolium	α-Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, *p*-cymene, α-ocimene, γ-terpinene, terpinolene, camphor, 2-pinene-4-ol [=(*Z*)-verbenol)], (**343**), α-terpineol, *p*-menth-1-en-8-ol, (*E*)-verbenol (**342a**), bornyl acetate, chrysanthenyl acetate (**48** or **49**)	Foods, beverages Antibacterial, antifungal, antiphlogistic, antiinflammatory, antipyretic, carminative, lower chest pain, depurative, diaphoretic, digestive, enema, febrifuge, heat rash symptoms,. hypocholesterolemic, ophthalmic, refrigerant, sedative, stomachache
Cicer arietinum (Chick pea)	*Seeds*: α-pinene, camphene, β-pinene, Δ^3-carene, myrcene, α-terpinene, limonene, β-phellandrene, γ-terpinene, *p*-cymene, terpinolene	Foods Antibilious, anticancer, anticonvulsant, antidiabetes, antidiarrheal, antiinflammatory, antinephrolithiasic, antioxidant, aphrodisiac, diuretic, estrogenic, hepatoprotective, hypocholesterolemic, hypolipidemic

(continued)

Table 1 (continued)

Cichorium endivia (Endive)	β-Pinene, *p*-cymene, (*Z*)-linalool oxide, (*E*)-linalool oxide, myrtenol, α-terpineol, carvone, dihydrocarvone, dihydroactinidiolide (**10**), actinidiolide (**2**), β-ionone, geranyl acetone	Vegetable Astringent For constipation, dyspepsia, snake bite
Cichorium intybus	1,8-Cineole, camphor, linalool, α-terpineol, thymol, carvacrol, geranyl acetone, β-ionone	Vegetable, food additives, beverages. *Root*: coffee substitute Antibacterial, antifungal, hypoglycemic For appetite loss, blood purifier, gall stones, gout, lowering blood sugar valance, maintaining hormonal swelling and skin irritation, premenstrual syndromes, rheumatism
Cinnamomum rivulorum	*Leaf oil*: α-pinene, geraniol, cumin aldehyde (**71**) *Bark oil*: geraniol, cumin aldehyde	Beverages
Cinnamomum camphora (Camphor oil)	α-Pinene, camphene, β-pinene, myrcene, α-phellandrene, limonene, camphor *Seed*: α-pinene, β-pinene, myrcene, limonene terpinolene, linalool, camphor, α-terpineol	Foods (young shoots and leaves cooked). Old leaves for flavoring in baked goods, candy, spice Anesthetic, anthelmintic, antifungal, antiinflammatory, antimicrobial, antineurotic, antioxidant, antirheumatic, antiseptic, antispasmodic, cardiotonic, carminative, diaphoretic, germicide, insecticide, sedative, stimulant, tonic, vermicide For digestive complaints and depression, decongestant, disinfectant For diarrhea, hysteria, nervousness
Cinnamomum camphora var. *glaucescens* (Apopino oil)	α-Pinene camphene, limonene, 1,8-cineole, linalool, camphor, α-terpineol, geraniol	Mosquito larvicidal, nematocidal
Cinnamomum cappru-corende	*Leaf oil*: linalool, geraniol, cumin aldehyde, cumin alcohol (**70**)	Beverages

(continued)

Table 1 (continued)

Cinnamomum cassia (Cassia oil)	*Cassia oil*: α-pinene, borneol, α-terpineol	Food flavor, beverages (herbal tea), cookies, bread, chewing gum, crude drug Analgesic, antibiotic, antiinfectious, antiinflammatory, antineuralgic, antiseptic, antispasmodic, antiviral, aphrodisiac, bactericidal, cholagogue, cicatrisant, decongestant, expectorant, digestive, emmenagogue, febrifuge, hepatic, hypotensive, insecticide, muscle relaxant, nervine, sedative, stimulant, stomachic, sudorific, tonic, vermifuge
Cinnamomum cassia	*Leaf/bark oil*: α-pinene, camphene, β-pinene, Δ^3-carene, myrcene, limonene, 1,8-cineol, (*E*)-β-ocimene, *p*-cymene, camphor, *iso*-borneol (**19a**), borneol, α-terpineol, geraniol, carvone, bornyl acetate	Beverages, flavoring agent Antiseptic For headache, neuralgia, stomach ulcer, diabetes, obesity
Cinnamomum citriodorum	α-Pinene, α-phellandrene, limonene, 1,8-cineol, linalool, (*E*)-rose oxide (**291**), *iso*-pulegol (**283**), citronellol, neral (**234a**), geraniol, geranial (**94**), α-terpinyl acetate, cumin aldehyde, cumin alcohol	Spice, beverages Stomach ailments
Cinnamomum dubium	*Leaf oil:* α-pinene, geraniol, cumin alcohol *Bark oil:* geraniol, cumin alcohol	Beverages
Cinnamomum loureirii (Japanese cassia oil)	α-Pinene, camphene, β-pinene, Δ^3-carene, limonene, 1,8-cineole, linalool, *p*-cymene, camphor, α-terpineol, citral	Food flavor for cookies, bread, chewing gum, spice, beverages

(continued)

Table 1 (continued)

Cinnamomum zeylanicum (Ceylon cinnamon oil)	α-Pinene, myrcene, limonene, *p*-cymene, α-phellandrene, 1,8-cineole, linalool, camphor, terpinen-4-ol, α-terpineol, geranyl acetate	Beverages, food flavor, spice, and flavor and aroma enhancer for beverage, cookies, bread, chewing gum, source Analgesic, anti-Alzheimer's disease, antiaging, antibiotic, antidiabetes, antifungal, antioxidant, antiparasitic, antiseptic, antispasmodic, aphrodisiac, astringent, bactericide, cardiac, carminative, emmenagogue, insecticide, nephropathy, stimulant, stimulating appetite, stomachic, tonic, vermifuge For diarrhea, cold, headaches, respiratory complaint, muscular aches and pain, stomach upset
Cinnamosma fragrans	1,8-Cineol, linalool, geraniol, nerol, geranic acid	Antimicrobial, anti-leishmania, antimultidrug resistant For malarial symptoms, fatigue, muscular aches
Cinnamosma macrocarpa	β-Pinene, limonene, γ-terpinene, terpinolene	Antiaging, anticancer, fatigue, isogenic, malarial symptoms, muscular aches
Cinnamosma madagascariensis	α-Thujene, α-pinene, camphene, sabinene, verbenene (**341**), myrcene, p-mentha-1(7),8-diene, limonene, α-phellandrene, β-phellandrene, α-terpinene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, (*E*)-β-ocimene, p-cymene, terpinolene, linalool, (*Z*)-linalool oxide, (*E*)-β-linalool oxide (**166**), α-campholenal (**28**), (*E*)-pinocarveol (**258a**), pinocarvone (**260**), borneol, α-phellandren-8-ol (=*p*-mentha-1,5-den-8-ol) (**270**), terpinen-4-ol, myrtenal, *p*-cymen-9-ol, myrtenol, verbenone, α-terpineol, cryptone, thymol methyl ether (**334**), carvacrol methyl ether (**35**), bornyl acetate, linalyl acetate (**168**), *iso*-bornyl acetate (**20**), myrtenyl acetate (**197**), α-terpinyl acetate (**311**)	For dementia, dental decay, elephantiasis, epilepsy, fatigue, filariasis, gastrointestinal, malarial symptoms, muscular aches, respiratory, parasitic infections

(continued)

Table 1 (continued)

Citrullus lanatus (= *C. vulgaris)* (Watermelon)	Limonene, neral, geranial, geraniol, geranyl acetone	Foods Anticancer, antiinflammatory, antioxidant For digestion, hair care, heart health, macular degeneration, muscle soreness
Citrus aurantifolia (Mexican lime oil)	α-Thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, α-terpinene, *o*-cymene, limonene, β-ocimene, γ-terpinene, α-terpinolene, linalool, fenchyl alcohol, *p*-menth-2-en-1-ol (**211a**), camphor, terpinen-1-ol (**306**), camphene hydrate (**24**), β-citronellal, borneol, isopulegone (**268**), terpinen-4-ol, geranial, neral, nerol, geraniol, bornyl acetate, citronellyl acetate, neryl acetate, geranyl acetate	Foods, cosmetic flavor. Antibacterial, anticancer, antidiabetes, antifungal, antihypertensive, antiinflammatory, antilipidemia, antioxidant For bone, heart, liver, urinary protection
Citrus aurantium ssp. *amara* (Bitter orange oil)	α-Pinene, β-pinene, myrcene, α-phellandrene, limonene, (*E*)-β-ocimene, α-terpinolene, linalool, neral, geranial, perillaldehyde, terpinen-4-ol, α-terpineol, linalyl acetate, geranyl acetate	Foods, beverages, cosmetics, crude drug (dried peel) Adrenergic, antibacterial, antiinflammatory, antiviral, cardiovascular and neurologic effects (dried peel)
Citrus aurantium subsp. *amara* (Petitgrain oil)	β-Pinene, camphene, limonene, linalool, geraniol, linalyl acetate	Flavor for soap, cosmetics Antimicrobial, antioxidant, antispasmodic
Citrus aurantium subsp. *amara* (Neroli oil)	α-Pinene, camphene, limonene, β-ocimene, (−)-linalool, α-terpineol, geraniol, linalyl acetate, geranyl acetate	Food flavor, beverages, flavor for cosmetics, compound flavor, bath additives, crude drug (dried peel) Antiinflammatory, antioxidant For anxiety, better sleep, depression, stress, stomachic, bitter stomachs

(continued)

Table 1 (continued)

Citrus aurantium subsp. *bergamia* (Bergamot oil)	α-Pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, β-phellandrene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, *p*-cymene, terpinolene, (*E*)-linalool oxide (**164a**, **165a**), (*Z*)-linalool oxide (**164b**, **165b**), (*E*)-limonene 1,2-oxide (**154a**), (*Z*)-limonene 1,2-oxide (**154b**), (*E*)-sabinene hydrate, terpinen-4-ol, citronellyl acetate, neral, α-terpinyl acetate, α-terpineol, neryl acetate, geranial, carvone, geranyl acetate, nerol, geraniol, perillyl acetate (**272**)	Foods. For many cosmetics: (eau de cologne, lotion, cream, soap) Antimicrobial, antiinflammatory For alleviates tension, anxiety, depression, digestion, hypotension
Citrus flaviculpus	α-Pinene, α-fenchene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, (*Z*)-β-ocimene, β-phellandrene, α-terpinene, *p*-cymene, γ-terpinene, terpinolene, α-thujone, (*Z*)-limonene oxide, menthone, (*E*)-linalool oxide, citronellal, camphor, terpinyl acetate, α-terpineol, neryl acetate, carvone, geranyl acetate, citronellol, perillaldehyde, carvone oxide (**44**), geranyl propanoate (**100**), *p*-menth-1-en-9-yl acetate, (**207**), nerol, (*E*)-carveol, *p*-menth-1-en-9-ol (**206**), *p*-menth-1,8-dien-9-ol or 10-ol (**218b**), limonene diol (**155**), neryl oxide (**241**)	Fruit, beverages, flavor, and fragrance
Citrus japonica	*Peel essential oil*: α-pinene, myrcene, limonene, β-phellandrene, carvone, geranyl acetate *Leaf essential oil*: 1,8-cineole, (*Z*)-linalool oxide, (*E*)-linalool oxide, linalool, camphor, borneol, δ-terpineol, menthol, terpinen-4-ol, (*E*)-*iso*-citral (**113a+232**), α-terpineol, citronellol, nerol, geranial, geraniol, bornyl acetate, geranyl formate, citronellyl acetate	Foods, fruit, beverages, jam, syrup, marmalade Antiphlogistic, antiviral, carminative, deodorant, expectorant

(continued)

Table 1 (continued)

Citrus junos (Yuzu)	α-Pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, *p*-mentha-1(7),8-diene (**201**), α-terpinene, limonene, β-phellandrene, γ-terpinene, *p*-cymene, terpinolene, *p*-mentha-1,4,8-triene (**216**), (*Z*)-β-ocimene, linalool, α-terpineol, (*Z*)-carveol, nerol, geraniol, perillyl alcohol, *p*-mentha-1,8-dien-10-ol (=limonene 10-ol) (**156**), (*Z*)-limonene oxide, (*E*)-limonene oxide, dehydro-*p*-cymene (**75**), (*E*)-sabinene-hydrate, thymol methyl ether, terpinen-4-ol, *p*-mentha-1(7),2-dien-8-ol (**213**),(7*E*)-8-hydroxy-linalool (**159a**)	Fruit, foods, beverages (herbal tea, liquor, wine), flavor, food additives, vinegar, flavoring many snack products, marmalade, cake, syrup, spicy Japanese sauce with green or red chili pepper, citrus bath (yuzu bath) Antihypercholesterolemia, antiobesity, antioxidant, α-amylase and lipase inhibitory
Citrus lemon (Lemon oil)	α-Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, γ-terpinene, limonene, linalool, neral, geranial, geraniol, neryl acetate, methyl geranate	Foods and food flavor, beverages. For many cosmetics; soap, eau de cologne, cosmetic water Bactericidal, stomachic
Citrus medica var. *sarcodactylis*	*Fruits/leaves*: α-pinene, β-pinene, myrcene, limonene, γ-terpinene, linalool, citronellal, neral, geranial, nerol, geraniol	Foods, beverages, sweets, Kampo medicines
Citrus medica var. *corsican*	α-Thujene, α-pinene, sabinene, β-pinene, myrcene, α-terpinene, *p*-cymene, limonene, (*E*)-β-ocimene, (*Z*)-β-ocimene, γ-terpinene, terpinolene, linalool, citronellal, *iso*-neral (**233**), *iso*-geranial, terpinen-4-ol, nerol, neral, geraniol, geranial, neryl acetate, geranyl acetate *Fruits/leaves*: α-pinene, β-pinene, myrcene, limonene, γ-terpinene, linalool, citronellal, neral, geranial, nerol, geraniol	Foods, beverages, sweets, Kampo medicines Analgesic, anthelmintic, antiinflammatory, antioxidant For cardiovascular health, constipation, diarrhea
Citrus paradisi (Grapefruit oil)	α-Pinene, β-pinene, sabinene, myrcene, α-phellandrene, limonene, (*E*)-ocimene, β-phellandrene, γ-terpinene, β-ocimene, linalool, (*Z*)-linalool oxide, (*E*)-linalool oxide, terpinen-4-ol, α-terpineol, neral, carvone, geranial, geraniol, geranyl acetate, perillaldehyde	Food flavor, beverage aroma Antiinflammatory, antiobesity, antistress, hypotension For acne, cold, depression, digestion, hangovers, headaches, jet lag

(continued)

Table 1 (continued)

Citrus reticulata var. *mandarin* (Mandarin oil)	α-Pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, β-phellandrene, (*Z*)-β-ocimene, γ-terpinene, *p*-cymene, terpinolene, (*E*)-sabinene hydrate, linalool, citronellal, terpinen-4-ol, neral, α-terpineol, nerol, geraniol, geranial, citronellal, geranyl acetate, citronellol, perillaldehyde, thymol, (*E*)-*p*-mentha-2,8-diol	Food flavor: soft drink, liquor, candy, compound fragrance Antiarteriosclerosis, antifungal, antiinflammatory, antioxidant For appetite, bilious diarrhea, carminative, catarrh, ear ache, fever, laxative, stomachic, tonic, toothache
Citrus sinensis (Orange oil)	α-Thujene, α-pinene, β-pinene, myrcene, limonene, α-terpinene, (+)-linalool, α-terpineol, geranial, neral, geraniol, nerol, citronellol, perillaldehyde	Foods, beverages, perfume, fragrances, cosmetics, crude drug (dried peel) Anticancer, antimicrobial, antioxidant, anxiety, depression, insecticide
Citrus sphaerocarpa	α-Thujene, α-pinene, β-pinene, sabinene, Δ^3-carene, myrcene, α-phellandrene, α-terpinene, limonene, β-phellandrene, γ-terpinene, *p*-cymene, terpinolene, dihydro-*p*-cymene, terpinene-4-ol, (*E*)-*p*-mentha-2,8-dien-1-ol, (*E*)-verbenol (**342**), (*E*)-piperitol (**262**), limonene-4-ol (**157**), terpinolene, γ-terpineol, piperitone, geranial, neryl acetate, geranyl acetate (**99**), β-citronellol, nerol	Fruit, beverages (juices, non-alcoholic and alcoholic), foods (frozen desserts, snack foods), flavor for cooked fish, Sashimi, soup, noodles), vinegar
Citrus sudachi	α-Thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, Δ^3-carene, limonene, α-phellandrene, *p*-mentha-1,(7) 8-diene (**202**), α-terpinene, *p*-cymene, ocimene, α-terpinene, terpinolene, 1,8-cineole, β-phellandrene, (*Z*)-β-ocimene, (*E*)-β-ocimene, linalool, (*E*)-sabinene hydrate (**296**), (*Z*)-linalool oxide, (*E*)-linalool oxide, β-terpineol, citronellal, pinocarveol, terpinen-4-ol, terpinolene-4,8-oxide (**318**), α-terpineol, dihydrocarvone, dihydrocarveol (**37**), (*Z*)-carveol, (*E*)-limonene-1,2-	Fruit, beverages (herbal tea, liqueur), food additives, flavor (cooked fish, Sashimi, soup, noodles), vinegar (Sushi, Tempura, Tofu) Antiinflammatory, antioxidant, hypocholesterolemic, antihypertension

(continued)

Table 1 (continued)

	oxide, (*Z*)-limonene-1,2-oxide, citronellol, (*E*)-carveol, (*E*)-*p*-mentha-2,8-dien-1-ol, cryptone, limonene-4-ol, cumin aldehyde (**71**), carvone, piperitone, *p*-menth-3-en-2-one (=*iso*-piperitone) (**282**), geranial, geraniol, perillaldehyde, perilla alcohol, citronellyl acetate, neryl acetate, piperitone, (*Z*)-*iso*-piperitenol (**280**), (*E*)-*iso*-piperitenol (**279**), β-*iso*-geraniol (**113b**), myrtenol, (*Z*)-*p*-mentha-1(7),8-dien-2-ol (**203b**), nerol, *iso*-piperitenone (**276**), *p*-cymen-9-ol, *p*-mentha-1,3-dien-7-ol (**215**), limonen-10-ol (**156**), cumin alcohol (**70**), thymol, carvacrol, geranyl acetone	
Citrus tamurana	α-Pinene, α-fenchene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, β-phellandrene, (Z)-β-ocimene, α-terpinene, *p*-cymene, terpinolene, α-thujone, (*Z*)-limonene oxide, menthone, (*E*)-linalool oxide, citronellal, camphor, terpinyl acetate, α-terpineol, neryl acetate, carvone, geranyl acetate, citronellol, perillaldehyde, carvone oxide (**44**), geranyl propanoate, *p*-menth-1-en-9-yl acetate (**207**), isopiperitone (**282**), nerol, (*E*)-carveol, *p*-menth-1-en-9-ol (**206**), *p*-menth-1,8-dien-10-ol (=limonene-10-ol) (**156**), limonene diol (**155**), nerol oxide (**241**)	Fruits, beverages
Citrus unshiu	Limonene, γ-terpinene, linalool, myrcene, *o*-cymene	Fruit, beverages (herbal tea), food additives Antimicrobial, antioxidant
Citrus yuko	*αγ*-Pinene, camphene, β-pinene, limonene, 1,8-cineole, terpinolene, α-terpineol, terpinen-4-ol	Foods, fruit, beverages, food additives, vinegar Antibacterial, antidiabetes, anticancer, antihypertension, antiobesity, antioxidant

(continued)

Table 1 (continued)

Clerodendrum trichotomum	Linalool	Foods (leaves) Analgesic, antihypertensive, antiinflammatory, antioxidant, antirheumatic, sedative
Clitocybe odora	Limonene, linalool	Foods
Coix lacryma-jobi	*	Foods, herbal medicines. Antiallergic, anticancer, antidiabetes, antiinflammatory, hypoglycemic, hypolipidemic, gastroprotective
Colchicum autumnale	*	For familiar Mediterranean fever, gout, narrow therapeutic index
Colocasia esculenta (rhizome) (Taro)	(*E*)-Linalool oxide, linalool, α-terpineol, nerol, geraniol, geranyl acetone, β-damascenone	Vegetable Anticancer, antihypertension For anemia, blood, bone and eye health, immune system booster
Conocephalum conicum	α-Pinene, camphene, β-pinene, limonene, terpinolene, camphor, linalool, bornyl acetate	Foods Antifungal, antimicrobial
Copaifera reticulata (Copaiba oil)	α-Pinene, β-pinene, Δ^3-carene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, γ-terpinene	Fragrance, perfume retention agent Analgesic, antibacterial, anticancer, antifungal, antihistamine, antiinflammatory, antimicrobial, antioxidant, antirheumatic, antitussive, antiviral, COX-inhibitor, depurative, febrifuge, hypocholesterolemia, insecticidal, modulator, pediculicide, purgative, tonic, vermifuge For immune system booster
Coptis japonica	α-Pinene, 1,8-cineol, citral	Beverages (Kampo medicine) For angina, bacterial bloody bowel discharge, diarrhea, hematemesis, mental anxiety, stomachache, stomatitis
Coriandrum sativum	α-Pinene, β-pinene, myrcene, *p*-cymene, limonene, camphene, α-terpinene, linalool, (*E*)-linalool oxide, *iso*-borneol, α-terpineol, cumin aldehyde, β-citral, geraniol, linalool, terpineol, citronellol, geraniol, geranyl acetate	Vegetable, flavoring of sources, liquor, spice for soup, curries Antianxiety symptoms, antimicrobial, antiglycemic, hypotension, increasing HDL cholesterol For digestive symptoms, reduce blood sugar

(continued)

Table 1 (continued)

	Seed essential oil: α-pinene, camphene, sabinene, *p*-cymene, linalyl acetate, limonene, γ-terpinene, (*E*)-β-ocimene, terpinolene, linalool, (*Z*)-β-sabinene hydrate, camphor, terpinen-4-ol, geraniol, geranyl acetate, α-terpineol, citronellol, nerol, myrtenyl acetate	Flavor for various commercial foods, as liquor, teas, meat products, and pickles Antibacterial, antifungal, antioxidant, antispoilage, hypoglycemic For joint pain, influence on carbohydrate metabolism
Coriandrum sativum	Linalool	Culinary herb Antifungal, antimicrobial For bowel spasms, digestion problems, diarrhea, hernia, hemorrhoids, intestinal gas, joint pain, toothaches, worms
Crataegus cuneata	α-Pinene, camphene, β-b-pinene, myrcene, Δ^3-carene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, β-ocimene, γ-terpinene, terpinolene, α-terpineol, *p*-menth-1-en-8-ol, linalool	Foods, beverages ACE inhibitory, antiarrhythmic, anti-HIV, antihyperlipidemic, antihypertensive, antiinflammatory, antimicrobial, antioxidant, cytotoxic, gastroprotective, hepatoprotective, hypoglycemic, radioprotective For cardiovascular disorders, central nervous and immune system, myocardial infarction, myocardial ischemia, congestive heart failure, eyes health
Crocus sativa (Saffron oil)	α-Pinene, β-pinene, 1,8-cineole	Fruit essence, spice, coloration for foods, deodorant Analgesic, sedative
Cryptotaenia japonica (Mitsuba)	α-Pinene, sabinene, β-pinene, myrcene, α-terpinene, limonene, γ-terpinene	Vegetable, flavor, for soup, pickles, stir fried Hemorrhages For cold, fevers, medicinal strengthened tonic, benefit in women
Cucumis melo	β-Pinene, 1,8-cineole, limonene, γ-terpinene, *p*-menth-1-en-8-ol (**205**), verbenone, β-cyclocitral, terpinyl acetate, geranyl acetone, β-ionone, dihydroactinidiolide	Fruit Antidiabetes, antihypertension For bone health, digestive disorders, eye health, immune function

(continued)

Table 1 (continued)

Cucumis sativus	Sabinene, β-pinene, limonene, (*E*)-β-ocimene, linalool, *p*-menth-1-en-3-one (**220**), α-terpinolene	Vegetable Antiaging, antidiabetes, antioxidant, blood protection, dyslipidemia
Cucurbita pepo (Pepo pumpkin)	α-Pinene, β-pinene, sabinene, myrcene, limonene, α-phellandrene, Δ^3-carene, α-terpinene, *p*-cymene, limonene, 1,8-cineole,. linalool, γ-terpinene, terpinen-4-ol, α-terpineol, β-cyclocitral, carvone, linalyl acetate, terpinyl acetate, β-ionone	Foods, beverages Antihypertension, antiinflammatory, anthelmintic For benign prostatic, erysipelas, headache, hyperplasia, irritable bladder, lower urinary tract symptoms, micturition disorders, neuralgia
Cucurbita maxima (Pumpkin)	α-Pinene, sabinene, β-pinene, myrcene, α-phellandrene, Δ^3-carene, limonene, α-terpinene, 1,8-cineole γ-terpinene, α-terpineol, linalool, geraniol, β-cyclocitral, carvone, linalyl acetate, α-terpinyl acetate, α-ionone, β-ionone	Vegetable Anticancer, antioxidant For cardiovascular and heart health, strengthening bone, improving vision
Cucurbita pepo var. *melopepo* (Zucchinis)	α-Pinene, myrcene, ocimene, limonene, 1,8-cineole, linalool, camphor	Beverages (herbal tea) vegetable Antiandrogen, antiinflammatory, reduction in prostate growth, detrusor activity
Curcuma aeruginosa	α-Pinene, camphene, β-pinene, 1,8-cineole, camphor, *iso*-borneol, borneol	Vegetable, food appetizers, dye, spice, food flavoring, coloring agent Antiasthma, antibronchitis, antifungal, antiinflammatory, antimicrobial, antinociceptive, antioxidant, antipyretic, antitumor, antiulcerogenic, cytotoxicity, dyspepsia, nitric oxide production, inhibitory, uterine relaxant For axillary, blood booster, cough, diarrhea, dysentery, fertilize the womb, flatulence, gastritis, hair growth stimulant, hemorrhoids, increase appetite, intestinal worms, etic infections, menstruation pain, parasitic infections, postpartum problem, skin penetration enhancer

(continued)

Table 1 (continued)

Curcuma amada	α-Pinene, β-pinene, (*Z*)-dihydroocimene (**253**), myrcene, (*E*)-dihydroocimene (**252**), α-phellandrene, *p*-cymene, carvomenthone (**41**), (*Z*)-β-ocimene (**250**), β-phellandrene, limonene, 1,8-cineole, (*E*)-β-ocimene (**251**), Δ^3-carene, α-terpinene, γ-terpinene, linalool, dihydromyrtenol (**199**), *p*-menth-4(8)-en-9-ol (**212**), *p*-menth-1-en-9-ol (**221d**), *p*-mentha-1,8-dien-9-ol (**218b**), bornyl acetate, citronellal (**55**), *p*-cymene-9-ol, camphor, camphene hydrate, β-terpineol, borneol, lavandulol, α-terpineol, geranial, neral, α-terpinyl acetate, thymol, α-ionone	Foods, flavor and spice, beverages Analgesic, antifungal, antiinflammatory, antimicrobial, antioxidant, antiplatelet, antipyretic, antitubercular, antitumor, antiulcer, CNS depressant, cytotoxic, expectorant, hepatoprotective, hypotriglyceridemia, insecticidal, insect repellent, larvicidal For aphrodisiac, appetizer, asthma, bronchitis, contusions, bruises diuretic, emollient, itching, laxative, rheumatic pain, skin diseases, sprains
Curcuma angustifolia	α-Pinene, camphene, myrcene, limonene, 1,8-cineole, (*E*)-linalool oxide, (*Z*)-linalool oxide, (*E*)-*p*-menth-2-en-1-ol (**211a**), camphor, camphene hydrate, *iso*-borneol, borneol, terpinen-4-ol, *p*-cymen-9-ol, myrtenal, α-terpineol, myrtenol, (*E*)-carvotanacetol (**45**), nerol, piperitone, geranial, *iso*-bornyl acetate (**20**), thymol, carvacrol, sabinyl acetate (**299**)	Food supplements, vegetable, food appetizers, cosmetic ingredient Analgesic, antifungal, antimicrobial, antioxidant, antiparasitic For asthma, body and gastrointestinal pain, dysentery, fevers, fungal infections, muscle relaxant, skin protecting
Curcuma aromatica	α-Pinene, camphene, β-pinene, myrcene, limonene, α-phellandrene, α-terpinene, 1,8-cineole, (*Z*)-β-ocimene, *p*-cymene, terpinolene, linalool, camphor, borneol, terpinen-4-ol, myrtenol, (*E*)-pinocarveol (**258a**), *iso*-borneol, α-terpineol, piperitenone (**275**)	Food supplements, beverages, flavoring, coloring agents, food appetizers Antiaging antiangiogenic, antibacterial, antidiabetes, antifibrotic, antifungal, antiinflammatory, antioxidant, antiplatelet, antiproliferative, antitumor, cardioprotective, carminative, cytotoxic, insecticidal, tonic For alleviating pain, blood stasis and circulation, complexion, chronic depression, liver diseases, infections, easing bruises, relieving sprains, skin eruptions, snake bites

(continued)

Table 1 (continued)

Curcuma australasica	*	Antiinflammatory, cytotoxic, nitric oxide and prostaglandin production inhibitory
Curcuma caesia	1,8-Cineol, camphor	Functional foods Antihepatotoxicity, antiinflammatory, antimicrobial, antioxidant, antitumor, antiulcer, muscle relaxant, carminative, dysentery, CNS depressant For asthma, bruises, constipation, cough, diarrhea, digestive problems, dysentery, epilepsy, jaundice, kidney disorders, leukoderma, piles, rheumatic pain, sprains, stimulant, skin and wound infection, tonsillitis, toothaches, urination
Curcuma domestica (=*C. longa*) (Curcuma oil or turmeric oil)	α-Pinene β-pinene, sabinene, myrcene, α-phellandrene, Δ^3-carene, limonene, α-terpinene, β-phellandrene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, (*E*)-β-ocimene, terpinolene, linalool, *p*-mentha-1,3, 8-triene, camphor, *iso*-borneol, borneol, *p*-cymen-8-ol, α-terpineol, terpinen-4-ol, verbenone, cumene, thymol, carvacrol, geranyl acetate	Food flavor, beverages, a dye, cosmetics α-Amylase and α-glucosidase inhibitor, anesthetic, anxiolytic, antiaflatoxigenic, antiallergic, anti-Alzheimer's disease, antiarthritic, antiatherosclerotic, antibacterial, anticarcinogenic, anticonvulsant, antidiabetes, antifatty liver, antifungal, antigenic, antihepatotoxic, antiinflammatory, antimutagenic, antiobesity, antioxidant, antiplatelet, antiproliferative, antithrombotic, antivasoconstrictive, antiulcerogenic, chemopreventive, cytotoxic, gastroprotective, hepatoprotective, hypoglycemic, hypothermic, hypolipidemic, immunomodulatory, insecticidal, insect repellent joint-protective, larvicidal, mosquitocidal (against *Aedes aegypti* and *Anopheles*), neuroprotective, neurotoxin-inhibitory, phytotoxic, scolicidal, sedative, spasmolytic, tonic

(continued)

Table 1 (continued)

		For anthelmintic, appetizer, carminative, chest congestion, cough, diabetic wounds, digestive aid, dysentery, infections, fever, gastritis, hypercholesterolemia, hypertension, jaundice, laxative, liver gall, bladder problems, menstrual discomfort, rheumatoid, skin diseases, stomachic, urinary tract infections
Curcuma glans	α-Pinene, β-pinene, limonene, 1,8-cineole, camphor, borneol, *iso*-borneol, *iso*-pinocamphone (**269**), terpinen-4-ol	Beverages Antibacterial, antiviral 4. For the herpes simplex virus, sore throat, tonsillitis, wounds or abscesses in the mouth, throat, nose
Curcuma haritha	Camphene, β-pinene 1,8-cineole, *iso*-borneol, camphor	Beverages
Curcuma harmandii	1,8-Cineol	Beverages
Curcuma harmandii	β-Pinene, 1,8-cineole	Beverages
Curcuma heyneana	α-Pinene, camphene, β-pinene, camphor, *iso*-borneol	Accelerating wound healing, antiinflammatory, antimicrobial, antioxidant For anthelmintic in skin scrub, body sliming, cancer prevention, diarrhea
Curcuma inodora	1,8-Cineole	Beverages
Curcuma kwangsiensis	1,8-Cineole, linalool, terpinen-4-ol, camphor, α-terpineol, *iso*-borneol	Food additives, herbal medicines Cytotoxic, antiinflammatory, antimicrobial, antioxidant
Curcuma leucorrhiza	1,8-Cineole, camphor, linalool, *iso*-borneol	Foods Antifungal, antimicrobial, antioxidant For cancer, cough, diabetes, treatment of enlarged liver and spleen, stomach ulcer
Curcuma mangga	β-Pinene, myrcene, *p*-menth-2-en-1-ol (**211a**), *p*-menth-1-en-9-ol, *iso*-pulegol	Foods Analgesic, antiallergic, anticoagulant, antifungal, antimicrobial, antioxidant, antiplatelet, antiproliferative, antitumor, antiulcerogenic, cytotoxic, nitric oxide production inhibition For ornamental, blood purifier, treatment swellings

(continued)

Table 1 (continued)

Curcuma phaeocaulis	α-Pinene, camphene, β-pinene, 1,8-cineole, linalool camphor *iso*-borneol, borneol, terpinen-4-ol, α-terpineol, verbenone, carvone, carveol	Food additives, beverages, herbal medicines Analgesic, antifungal, antiinflammatory, antimicrobial, antioxidant, antithrombotic, antiviral, cytotoxic For alleviating pain, hepatoprotective, gastritis, reducing blood stasis, tumor therapy
Curcuma pierreana	α-Pinene, camphene, 1,8-cineole, camphor, *iso*-borneol, bornyl acetate, *iso*-bornyl acetate	Food flavoring, vegetable, spice, dye, coloring agent, appetizers Antifungal, antimicrobial
Curcuma purpurascens	1,8-Cineol, camphor	Angiogenesis, antifungal, antioxidant, antiproliferative, apoptogenic, cytotoxic, gastroprotective
Curcuma rhabdota	Camphor	Beverages
Curcuma rubescens	Camphor	Beverages
Curcuma singularis	Camphor, fenchone	Antibacterial
Curcuma sylvatica	α-Fenchene	Beverages
Curcuma wenyuji	1,8-Cineole, linalool, terpinen-4-ol, α-terpineol, *iso*-borneol	Food additives. Antiinflammatory, antimicrobial, antioxidative, cytotoxic
Curcuma xanthorrhiza	α-Pinene, camphene, β-pinene, α-phellandrene, α-terpinolene, β-phellandrene, terpinolene, 1,8-cineole, γ-terpinene, terpinolene, camphor, *p*-cymen-8-ol (**80**), borneol, *iso*-borneol, *iso*-carveol (**40**), *iso*-pinocamphone (**269**), citronellyl pentanoate (**64**)	Foods, food additives, food coloring, spice, dye, cosmetics Analgesic, antiaflatoxigenic, antibacterial, anticancer, anticandidal, antihepatotoxic, antihyperglycemic, antihyperlipidemic, antiinflammatory, antimetastatic, antimicrobial, antimycotic, antinociceptive, antiobesogenic, antioxidant, antiulcerogenic, antispasmodic, antitumor, diuretic, estrogenic, hepatoprotective, hypotensive, gastroprotective, larvicidal, neuroprotective For gallstones, arthritis, Alzheimer's disease, dysentery, bloody diarrhea, bursitis, carpal tunnel syndrome, cataracts, chemopreventive, children's fevers, constipation, diabetes, eczema, endometriosis, gastric

(continued)

Table 1 (continued)

		problem, osteoporosis, halitosis, heart diseases, high blood pressure, hemorrhoids, hypertension, hypotriglyceridemia, liver cirrhosis, rheumatism, skin and stomach diseases, tendinitis, and skin eruptions
Curcuma zedoaria	1,8-Cineole, *p*-cymene, α-phellandrene, camphor, α-terpinyl acetate, *iso*-borneol, borneol	Foods, food additives, beverages Aggregation analgesic, antiallergic, antiangiogenic antifungal, antigingivitis, antihypertensive, antiinflammatory, antimicrobial, antimutagenic, antinociceptive, antiplatelet antiproliferative, antioxidant, antitumor antiulcerogenic, carminative, cytotoxic, diuretic, demulcent, expectorant, hemagglutinating, hypoglycemic, insecticidal, larvicidal, piscicidal, rubefacient, stimulant
Cydonia oblonga (Marmelo)	β-Phellandrene, 1,8-cineole, (*Z*)-linalyl oxide, camphor, α-terpineol, β-cyclocitral, (*E*)-damascone, *iso*-citral [= *iso*-geranial (**113a**) + *iso*-neral (**232**)]	Foods, fruit, beverage (cider, wine fruit in liquor), jam, jelly, marmalades, pastes, pudding Antiallergenic, anticancer, antidiabetes, antiinflammatory, antioxidant, antivinous, astringent, cardiac, carminative, digestive, diuretic, emollient, expectorant, sedative, tonic For cardiovascular reproductive, gastric ulcers, gastrointestinal and coronary diseases, hair health, heart attacks, immune system booster, peptic, rectral, respiratory disorders, refrigerant, restorative, skin care, smooth muscle contraction, stimulant, stroke, treatment of dysentery, weight loss aid
Cymbopogon citratus	Myrcene, limonene, (*E*)-β-ocimene, (*Z*)-β-ocimene, α-terpinolene, citronellol, (*Z*)-verbenol, nerol, neral, geraniol, carveol, geranyl acetate	Flavoring agent in foods, drinks, and desserts Analgesic, antibiotic, antiinflammatory, antimutagenic, sedative For circulation, immunity booster, mosquito repellent

(continued)

Table 1 (continued)

Cymbopogon citratus	Citronellal	Incense, pesticide, perfume, carminative
Cymbopogon flexuosus (Lemongrass oil)	α-Pinene, camphene, β-pinene, myrcene, *p*-cymene, limonene, linalool, β-ocimene, γ-terpinene, α-terpineol, citral, citronellal, geraniol, geranyl acetate, geranic acid	Flavoring agent in foods Antibiofilm, anticancer, antifungal, antimicrobial, cytotoxicity
Cymbopogon nardus (Ceylon *Citronera* oil)	Camphene, (−)-borneol, citronellal, geraniol, citral, citronellal, (+)-citronellol, geranyl acetate	Food flavoring, perfume, beverages Analgesic, antimicrobial, antiparasitic, diuretic, insecticide For strengthening muscle spasms, increasing appetite
Cymbopogon winterianus (Java *Citronera* oil)	Limonene, linalool, isopulegol, citronellal, neral, geraniol, geranial, citronellyl acetate, neryl acetate	Flavor ingredient Antimicrobial, antiseptic, antispasmodic, febrifuge
Cynanchum rostellatum (=*Metaplexis japonica*)	Linalool, α-terpineol	Beverages (herbal tea), tonic For mental fatigue
Cynara scolymus (Artichoke)	Limonene, myrtenal. verbanol (**339a**), carvone, thymol	Vegetable Antiulcer For hypercholesterolemia, regulating blood pressure, improving liver and digestive health, lowering blood sugar, symptoms of irritable bowel syndrome
Cupressus sempervirens var. *stricta* (Cypress oil)	α-Pinene, camphene, sabinene, β-pinene, myrcene, Δ^3-carene, limonene, terpinolene, α-terpineol, bornyl acetate, α-terpinyl acetate	Compound flavor. Antispasmodic For regulating blood flow, aiding the respiratory system, relieving stress
Daucus carota (Carrot)	Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, α-phellandrene, β-phellandrene, (*E*)-α-ocimene, γ-terpinene, α-terpinolene, *p*-mentha-1,3,8-triene, *p*-cymene, terpinen-4-ol, α-terpineol, camphor, borneol, *p*-cymen-9-ol, thymol methyl ether, β-citral, carvone, bornyl acetate, geranyl acetate, geranyl acetone, linalyl 3-methylbutanoate (=isovalerate) (**172**), *iso*-bornyl acetate, geranyl acetone, geranyl butanoate (**101**), geranyl isobutanoate (**102**), geranyl 2-methylbutanoate (**104**)	Vegetable Anticancer, antifungal, antimicrobial, antioxidant antiviral For blood circulation, stimulating nutraceutical, promoting hair growth, strengthening hair, follicle hair roots

(continued)

Table 1 (continued)

Dendrobium moniliforme	1,8-Cineole, terpinolene, linalool, terpinen-4-ol	Beverages Antiatopic dermatitis, anticancer, antifatigue, anticonstipation, antihypertension, antioxidant, antiproliferative, gastric hypoglycemic, hypolipidemic, immune enhancement, tonic For cure throat inflammatory, secretion of saliva, ulcer protective
Dianthus caryophyllus (Carnation oil)	Limonene, β-ocimene	Beverages (herbal tea), perfume, tonic for fevers For calming the nervous system, congestion, heart health, lowering fever and pain, mosquito repellent, nervousness and stress, skin health, sweat production, skin benefits
Dimocarpus longan	β-Myrcene, Δ^3-carene, α-phellandrene, *p*-cymene, *p*-menth-1,3,8-triene, *allo*-ocimene (**245**, or **246**), (*E*)-β-ocimene, camphor	Fruit Anticancer, antidote, antifungal, antiinflammatory, antioxidant, febrifuge, as a tonic for insomnia, neurasthenic, stomachic, vermifuge For anemia, anxiety, iron deficiency, insomnia stress, stomachic, febrifuge
Dioscorea alata (Water yam, greater yam) (cooked)	α-Pinene, β-pinene, myrcene, limonene, terpinolene, linalool, terpinen-4-ol, α-terpineol	Foods Laxative, vermifuge For fever, gonorrhea, inflamed hemorrhoids, leprosy, tumors
Dioscorea japonica (Japanese yam)	Camphor, menthol, borneol, terpein-4-ol, geraniol, geranyl acetone	Foods Hypercholesterolemia
Diospyros kaki (Persimmon)	β-Ionone, geranyl acetone	Foods, fruit, for salad, jam, dried fruit Acaricidal, anticancer, antihypertension, antifungal, antiinflammatory, antimicrobial, antioxidant, antitumor, hypocholesterolemic For boosting asthma, cough with phlegm, defecate, diarrhea, digestion, dysentery, eye disorders, health of lungs, spleen, heart, smooth up blood circulation, blood pressure, heart health

(continued)

Table 1 (continued)

Elettaria cardamomum var. *minus* (Cardamom oil)	α-Pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, limonene, 1,8-cineole, *p*-cymene, (*Z*)-β-ocimene, (*E*)-β-ocimene, linalool, *p*-cymen-9-ol, fenchene (**88**), fenchone (**92b**), limonene-1,2-epoxide, linalool oxide, borneol, α-terpineol, cryptone, camphor, pinocarvone, β-fenchyl alcohol (**91**), terpinen-4-ol, carvone, myrtenol, phellandral (**265**), thujyl alcohol (**326**), nerol, geraniol, citronellol, (*E*)-pinocarveol, linalyl acetate, *iso*-borneol, α-terpinyl acetate, α-fenchyl acetate, (**90**), β-fenchyl acetate (**92a**)	Food flavor, cookies, source, liqueurs, luxury spices Antifungal, antimicrobial, antiviral, carminative, stomachic, tonic For asthma, colic, constipation, diarrhea, diuretic, dyspepsia, epilepsy, hypertension, muscle pain
Eleutherococcus senticosus (=Acanthopanax senticosus)	α-Pinene, β-pinene, myrcene, *p*-cymene, β-phellandrene, linalool epoxide, terpinen-4-ol, cryptone, α-terpineol	For angina pectoris, cold, coronary heart disease, diabetes, Helpes simplex, inflammation, treatment of combined neurosis, lowering blood sugar, cold, coronary stress-induced pathophysiologic changes, menopausal syndrome, strengthening the spleen and kidney, nourishing the kidney
Enteromorpha prolifera (Green laver)	α-Pinene, limonene, terpinolene, linalool, 1,8-cineole, carvone, geraniol, β-ionol (**116**), dihydroactinidiolide	Sea vegetable (for snacks) Antioxidant
Equisetum arvense	Linalool, α-ionone (**120**), dihydroactinidiolide (**3**), 3-oxo-α-ionone (**117**), 4-oxo-α-ionone (**119**)	Food (sporophytes) Antibacterial, antiinflammatory, antimicrobial, antioxidant, astringent, coagulant, demulcent, diuretic

(continued)

Table 1 (continued)

Eriobotrya japonica (Loquat)	Cumene, α-thujene, α-pinene, camphene, β-pinene, limonene, 1,8-cineole (*Z*)-β-ocimene γ-terpinene, α-terpineol, linalool, (*Z*)-β-terpineol (**310**), terpinen-4-ol, *p*-cymen-8-ol (**81**), α-terpineol, geraniol, (*E*)-β-ionone	Fruit, beverages, allaying vomiting and thirst, expectorant, sedative *Leaves*: Analgesic, antibacterial, antiemetic, antitussive, antiviral, astringent, coughs, feverish colds, diuretic, expectorant, intestinal astringent, mouthwash bronchitis *Flower*: Expectorants *Fruit*: Allaying vomiting and thirst, expectorant, sedative
Eucalyptus citriodora (*E. citriodora* oil)	α-Phellandrene, 1,8-cineole, citronellal, citronellol, citronellyl acetate, geranyl acetate, *iso*-pulegol (**283**), *neoiso*-pulegol (**284**)	Disinfectant, compound fragrance, perfumery Insect repellent
Eucalyptus dives (Piperitone-phellandrene-eucalyptus oil)	α-Thujene, camphene, α-phellandrene, limonene, 1,8-cineol, *p*-cymene, piperitone (**281**)	Disinfectant, deodorant Antifungal, antiinflammatory, antimicrobial For allergies, cough, earaches, headaches, improving respiratory conditions, boosting mental clarity, and aiding wound care
Eucalyptus globulus (Eucalyptus oil)	α-Pinene, camphene, 1,8-cineole, pinocarveol, pinocarvone, myrtenol, verbenone, carvone	Deodorant Bactericide, insecticide For disinfectant, oral care drugs
Eucalyptus macarthurii (Geranyl acetate-eucalyptus oil)	Geraniol, linalool, geranyl acetate	Flavoring, backed goods, confectionary, meat products, beverages, compound fragrance
Eucommia ulmoides	β-Cyclocitral, geraniol, β-damascone, α-ionone, geranyl acetone	Anticancer, antihypertension, antisenescence For debasing fattiness, diuretics, enhancing the strength, preventing miscarriage
Eugenia caryophyllus (Clove oil)	α-Thujene, α-pinene, β-pinene, myrcene, α-terpinene, fenchone, geranial (**94**), cumin aldehyde, α-terpinyl acetate	Food flavor, spice, high-grade cosmetics, oral care drugs, digestive ailments Antifungal, antimicrobial For diarrhea, intestinal worms
Eupatorium japonica	α-Pinene, camphene, β-pinene, myrcene, (*Z*)-β-ocimene, (*E*)-β-ocimene, bornyl acetate	Antipyretic, diuretic, hematemesis, neuralgia For edema

(continued)

Table 1 (continued)

Evernia prunastri (Oak moss oil)	(−)-α-Pinene, (−)-camphene, (−)-β-pinene, myrcene, limonene, *p*-cymene, 1,8-cineol, linalool, camphor, (−)-carvone, (+)-α-thujone, (−)-β-thujone, (+)-terpinen-4-ol, (+)-α-terpineol, (−)-borneol, α-fenchyl alcohol, β-fenchyl alcohol, geraniol, citronellol	Perfume fixative Antiinflammatory, antiirritant, antimicrobial, antiseptic, expectorant
Fagopyrum esculentum (Buckwheat)	*Boiled powder*: α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, linalool, β-cyclocitral, menthone, *p*-cymene, camphor, α-terpinyl acetate, linalool oxide, terpinen-4-ol, nerol, geraniol, bornyl acetate, geranyl acetone, thymol, methyl geranate, dihydroactinidiolide	Foods, raw or cooked, noodles, pancakes, breads Capillary tonic For eczema, erysipelas, high blood pressure, gout, liver disorders, strengthening blood vessel, radiation damage, varicose veins
Fallopia japonica	α-Pinene, β-pinene, linalool, β-ionone epoxide	Anticancer, antihypertension, antiinflammatory, antitussive, diuretic For cognitive problems, gastrointestinal health, heart ailments, hemostatic improvement of cystitis, irregular menstrual periods
Farfugium japonicum (=*Ligularia tussilaginea*)	α-Pinene, sabinene	Vegetable, salads, soups, beverages (seed) Anticancer, antiinflammatory, antimicrobial, antioxidant
Ferula assa-foetida	α-Pinene, camphene, sabinene, β-pinene, myrcene, *p*-cymene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene	Foods, candies Antispasmodic, carminative, expectorant, laxative, sedative Stimulant, emmenagogue, vermifuge, blood, and lowers blood pressure For asthma, bronchitis, flatulent, colic, hysteria, infantile pneumonia, whooping cough
Ficus carica	α-Pinene, camphene, β-pinene, Δ^3-carene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, linalool, (*Z*)-menth-2-en-1-ol, (*E*)-menth-2-en-1-ol, (*E*)-verbenol, terpinen-4-ol, α-terpineol, (*Z*)-piperitol,	Foods Abortifacient, analgesic, antiarthritic, diuretic, emetic, febrifuge, stimulant, tonic, vermifuge

(continued)

Table 1 (continued)

	verbenone, carvacrol methyl ether, linalyl acetate, bornyl acetate, terpinen-4-yl acetate, (*E*)-piperityl acetate (**264**), α-terpinyl acetate, geranyl acetate, β-cyclocitral, (*E*)-β-damascenone (**84**), (*E*)-β-damascone	
Fistulina hepatica	α-Pinene, β-pinene, limonene, 1,8-cineole, linalool, menthol, α-terpinene, (*E*)-geranyl acetone	Foods
Flammulina velutipes	β-Pinene, α-terpinene, dihydrocarveol	Foods
Flammulina velutipes (Winter mushroom)	α-Pinene, sabinene, limonene, α-terpinene, *p*-cymene, camphor, menthofuran (**182a**), *p*-menthen-3,9-epoxide (**182b**) *Heated material*: limonene, sabinene, α-terpinene	Foods Anti-Alzheimer's disease, anticancer, anticholesterolemic, antiinflammatory, hypolipidemic For cholesterol and lipid reduction, dementia, gastric ulcers, hypertension, liver disorders, thrombosis
Foeniculum vulgare	α-Phellandrene, fenchone, thymol, carvacrol	Foods, flavorings in baked goods, meat and fish dishes, ice cream, alcoholic beverages, and herb mixtures Acaricidal, antidiabetes, antifungal, antihirsutism, antiinflammatory, antimicrobial, antioxidant, antithrombotic, antitumor, cytochrome inhibitory, cytoprotection, estrogenic, hepatoprotective, uterine contraction
Fragaria elatior (Strawberry oil)	Verbenone, citronellol, α-terpineol, myrtenol, carveyl acetate	Foods, concentrated juice Antiallergic antiasthma, antidiabetes, antihypertension For enhancing cognitive function, heart protection, improving eye sight, strengthening immune system, treating arthritis and gout
Garcinia mangostana	Linalool, α-terpineol, limonene, (*E*)-β-ocimene, (*Z*)-β-ocimene, linalool oxide, linalool, neral, nerol, geraniol	Antimicrobial, antiobesitic, antioxidant For eczema, burn, sores, thirst for one's mouth

(continued)

Table 1 (continued)

Gardenia jasminoides	Linalool, terpineol, linalyl acetate	Natural yellow dye. Analgesic, antioxidant, antiseptic, antispasmodic, diuretic, hypotensive, sedative For abdominal pain, carminative, cold, cough, dyspepsia, fever, flatulence, hepatitis, lymph node, menorrhagia, tuberculosis
Gaultheria miqueliana (Wintergreen oil)	α-Thujene, α-pinene, sabinene, myrcene, ocimene, carene, α-phellandrene, limonene, *p*-mentha-1(7),2-diene (**214**), *p*-mentha-1,8(9)-diene, 1,8-cineole, 1inalool, bornyl acetate, neral, geraniol, citronellol, methyl geranate, fenchone, menthone	Flavor, candy, chewing gum, soft drink Antiinflammatory, antiseptic For cold, detoxification, digestion, fever, flu, headache, infections, muscle relaxation
Ginkgo biloba	α-Pinene, limonene, α-ionone, β-ionone (**122**)	Food (seed), beverage. Alzheimer's disease, antiaging, anticancer, antihypertension, antiinflammatory For anxiety, asthma, cancer prevention, dementia, depression, fibromyalgia, hemorrhoids, heart health, hypertension, multiple sclerosis, tinnitus, vertigo
Glechoma hederacea	α-Pinene, β-pinene, sabinene, myrcene, 1,8-cineol, (*E*)-β-ocimene, (*Z*)-β-ocimene, *p*-mentha-2,4(8)-diene (**208**), *allo*-ocimene, pinocamphone (**261**), *iso*-pinocamphone (**269**), myrtenal	Beverages Antiphlogistic, antimutagenic, antioxidant, expectorant For anodyne, digestive, stimulant
Glehnia littoralis	α-Pinene, β-phellandrene, geraniol	Beverages (herbal tea) Aphrodisiac, antitussive, expectorant For asthma, cough
Glycine max (Soybeans)	Linalool, (*Z*)-jasmone	Foods, beverages Anticancer, antiinflammatory For birth defects, bone health, diabetes, digestion, heart health, healthy weight gain, metabolic alleviation of menopausal symptoms, obesity, sleep disorders

(continued)

Table 1 (continued)

Glycyrrhiza echinata	Tetrahydrolavandulol (**141**)	Beverages (herbal tea), sweet ingredients Expectorant, tonic
Glycyrrhiza glabra	(*Z*)-Pinene hydrate (**287**), tetrahydrolavandulol, terpinen-4-ol, (*E*)-linalool oxide, *p*-cymen-9-ol, α-terpineol, cumin aldehyde (**71**), piperitone, thymol, carvacrol	Food Antiinflammatory For arthritis, congestion, diabetes, fatigues, infertility, menopause, menstrual disorder, mucous nausea, poor circulation, respiratory infection, rheumatoid, tonsillitis, ulcer
Glycyrrhiza inflata	Tetrahydrolavandulol, cumin aldehyde, carvacrol	Cosmetics
Glycyrrhiza uralensis	Thujene, linalool, α-terpineol, carvacrol, *p*-cymene, fenchone, lavandulol, *p*-cymenol (**83**), linalool oxide	Natural sweeter, candies
Grifola frondosa	(*E*)-Sabinene hydrate, (*Z*)-sabinene hydrate	Foods
Guaiacum officinale (Guaiac wood oil)	*	Flavor, compound flavor materials, chewing gum and cakes Antimicrobial For arteriosclerosis, chronic, cold, cough, cutaneous diseases, dysmenorrhea, flu, poor circulation, respiratory complaints, rheumatism, toothache
Gymnema sylvestre	1,8-Cineole	Dietary supplements. Antiinflammatory, antimicrobial, antioxidant, diuretic For anemia, arthritis, asthma, cardiopathy, constipation, diabetes, hypercholesterolemia, indigestion, microbial infections, osteoporosis
Gynostemma pentaphyllum	1,8-Cineole	Beverages (herbal tea) Antioxidant, appetite enhancement
Hamamelis virginiana	Linalool, (*Z*)-linalool oxide, (*E*)-linalool oxide, myrtenol, (*E*)-pinocarveol, nerol oxide, *iso*-borneol, terpinen-4-ol, *p*-cymen-9-ol, α-terpineol, myrtenol, nerol, *iso*-bornyl formate (**19b**), geraniol, geranyl formate (**98**), geranyl acetate, (*E*)-β-damascenone	Beverages Antiinflammatory, antiviral For bruise, gastric ulcer, hematemesis, hemorrhages, hemorrhoids, prostration, sore throats, stool, traumatic conjunctivitis

(continued)

Table 1 (continued)

Helianthus annuus (Sunflower)	Roasted seed: carvacrol, thymol, sabinene hydrate	Foods Antioxidant
Heliotropium peruvianum	α-Pinene, camphene, Δ^3-carene, limonene, (*E*)-β-ocimene, *p*-cymene, linalool, thujone	Beverages, high-grade fragrance, food flavor; cookies, candy Antiphlogistic, diuretic For curative vulnerary, malaria, wound healing
Hordeum vulgare (Barley)	(*E*)-Damascenone	Food grain, natural sweeter, brewing beer, alcoholic beverages Antidigestion, antihypertension, antiinflammatory, hypocholesterolemic, hypoglycemic, weight loss promotion For bronchitis, diabetes, febrifuge, indigestion
Houttuynia cordata	α-Pinene, camphene, β-sabinene, β-pinene, myrcene, 1,8-cineol, *p*-cymene, linalool, α-terpinene, limonene, (*E*)-β-ocimene, geraniol, bornyl acetate, geranyl acetate, α-cyclogeranyl acetate (**114**), thymol	Foods, nutrient, beverages Antibacterial, anticancer, antiinflammatory, antimutagenic, antiviral (HSV-1, HIV-1), immunologic function
Hygrophorus agathosmus	α-Pinene, β-pinene, 1,4-cineole (**51**), limonene, 1,8-cineole, *p*-cymene, linalool, α-terpineol, geranyl acetone, β-ionone	Foods
Hypericum perforatum (St. John's wort)	α-Pinene, β-pinene, α-fenchene, myrcene, α-phellandrene, *p*-cymene, limonene, (*E*)-β-ocimene, α-terpinene, (*Z*)-thujone, β-fenchyl alcohol, (*Z*)-*p*-menth-2-en-1-ol (**211b**), α-campholenal (**28**), (*E*)-pinocarveol (**258a**), camphor, *iso*-pulegol, *iso*-borneol, (*E*)-pinocamphone, pinocarvone, *p*-cymen-9-ol, α-terpineol, myrtenol, verbenone, (*E*)-carveol, carvone, geranial, geranyl acetone	Beverages (herbal tea) Antibacterial, antifungal, sedative, analgesic, diuretic, antimalarial, vulnerary For burns, contusions, depression, gastroenteritis, hemorrhoids, hysteria, neuralgia, sprains, rheumatism, trauma, ulcers

(continued)

Table 1 (continued)

Hyssopus officinalis	α-Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, β-phellandrene, (*Z*)-β-ocimene, α-thujene, β-thujene, pinocamphone, *iso*-pinocamphone, linalool, pinocarvone, myrtenol	Pungent spice Diaphoretic, expectorant, mild stimulant, vulnerary poultice
Illicium verum (Star anise oil)	α-Pinene, β-pinene, limonene, phellandrene, 1,8-cineole, linalool	Spice, fragrant oil for cooking, perfumery, soaps Liqueur brandy, cookie flavor attached, crude drugs Carminative, mild stimulant For carminative, toothpastes, mouthwashers, skin cream, stomachic
Ipomoea batatas (Sweet potato)	*Grilled:* linalool, geraniol, β-ionone *Head space*: linalool, β-ionone *Alcohol beverage (Imo Shochu)*: α-terpineol, citronellol, nerol, geraniol	Vegetable, alcoholic beverages Antiinflammatory For arthritis, asthma, bronchitis, diabetes, digestion, cancer, immunity booster, stomach ulcer
Iris germanica (= *I. florentina*) (*Iris* resinoid)	α-Pinene, β-pinene, linalool, geraniol	Beverages, deodorant for liquor, whiskey, wine Expectorant For breath freshener, bronchitis, congestion, cough, decongestant, dropsy, sore throat
Jasminum officinale *Jasminum officinale* var. *grandiflorum* (Jasmine oil)	(+)-Linalool, linalyl acetate, nerol, geraniol, jasmone (**133**), methyl jasmonate (**134**), jasmine lactone (**132**)	Beverages, food flavor, high-grade compound fragrance Antioxidant, antifertility, antiviral, dermatological, emmenagogue, insecticidal, sedative For aphrodisiac, cough, insomnia, skin care
Jasminum sambac	Linalool, linalyl acetate	Beverage, flavoring ingredients in jasmine tea Antifungal, antimicrobial, antioxidant
Juglans mandshurica	α-Pinene, sabinene, β-pinene, (*E*)-β-ocimene, terpinolene, linalool, pinocarvone, pinocarveol, myrtenal, myrtenol, verbenone, verbenol	Foods Antiinflammatory, anticancer For diabetes, cardiovascular disease, bone metabolism, sleep cycle health
Juglans mandshurica var. *sachalinensis*	α-Pinene, sabinene, β-pinene, (*E*)-β-ocimene, terpinolene, linalool, pinocarvone, pinocarveol, myrtenal, myrtenol, verbenone, verbenol	Foods Antiinflammatory, anticancer For diabetes, cardiovascular disease, bone metabolism, sleep cycle health

(continued)

Table 1 (continued)

Juniperus utilis	α-Pinene, myrcene, Δ^3-carene, β-pinene, α-terpineol, borneol citronellol, bornyl acetate, α-terpinyl acetate, citronellyl acetate	Foods, condiments, beverages, flavoring ingredients, source of gin and medicinal liquor Antimicrobial, digestion, removal of kidney and bladder stones
Juniperus virginiana (Cedar wood oil)	Tricyclene, α-pinene, camphene, fenchene, thuya-2,4 (10)-diene (**323**), β-pinene, sabinene, β-phellandrene, α-terpinene, *p*-cymene, limonene, (*Z*)-β-ocimene, γ-terpinene, terpinolene, (*Z*)-sabinene hydrate (**297**), (*Z*)-linalool oxide, (*Z*)-rose oxide (**292**), (*Z*)-*p*-menth-2-en-1-ol, α-campholenal (**28**), geijerene (**93**) (*E*)-pinocarveol, camphor, (*Z*)-verbenol (**343**), (*E*)-verbenol (**342a**), (*E*)-pinocamphone (**261**), terpinene-4-ol, α-terpineol, myrtenal, verbenone, (*E*)-carveol, carvacrol methyl ether, bornyl acetate, carvacrol, citronellyl acetate, (*E*)-myrtenyl acetate (**197**)	Compound flavor, soap flavor, inner aroma Antiseborrheic, antiseptic, antispasmodic, astringent, diuretic, emmenagogue, expectorant, insecticide sedative, tonic
Lagenaria siceraria var. *gourda* (Bottle gourd)	Limonene, Δ^3-carene, *p*-menth-1-en-3-one (**221b**), terpinolene, geranyl acetone	Foods, vegetable Adenopathy, alexiteric, alopecia, anticancer, antitumor, diuretic, dropsy, emetic, insanity, laxative, lithontripic, purgative, rheumatic For ache, bilious, burn, convulsion, fever, wound
Laminaria japonica (=*Saccharina japonica*) (Kelp)	α-Terpineol, β-cyclocitral, β-homocyclocitral (**74**), β-ionone	Foods, beverages Anticancer, antiinflammatory, antioxidant, hypocholesterolemic, hypoglycemic, lower cholesterol, immunity booster For digestion, menstruation symptoms

(continued)

Table 1 (continued)

Laurus nobilis (Laurel leaf oil)	α-Thujene, α-pinene, camphene, sabinene, β-pinene, α-phellandrene, Δ^2-carene, α-terpinene, o-cymene (**30**), 1,8-cineole, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, (*Z*)-sabinene hydrate, *p*-mentha-3,8-diene (**221b**), (*E*)-sabinene hydrate, linalool, *allo*-ocimene, (*E*)-sabinol, (**298**), camphor, β-pinene oxide (**288**), *iso*-borneol, *iso*-pulegol (**283**), *neoiso*-pulegol (**284**), α-terpineol, (*Z*)-carveol, (*Z*)-*p*-mentha-1(7),8-dien-1-ol, (*E*)-sabinene hydrate, sabinyl acetate (**299**), *neo*-3-thuyanol acetate (**325b**), *iso*-verbanol acetate (**340b**), α-terpinyl acetate	Flavor for meat products, soup, cookies, source, spice, beverages Antibiotic, antimicrobial, antineuralgic, antispasmodic, astringent, insecticide, sedative, tonic For bronchitis, flu
Lavandula angustifolia (=*L. vera*)	α-Pinene, camphene, myrcene, α-terpinene, *p*-cymene, 1,8-cineole, ocimene, γ-terpinene, sabinene hydrate, terpinolene, linalool, *iso*-pulegol, (*E*)-pinocarveol, camphor, borneol, linalool oxide, terpinene-4-ol, *m*-cymen-9-ol (**81**), α-terpineol, (*Z*)-verbenone, eucarvone (**87c**), camphor, (**87c**), nerol, linalool hydrate (**163**), carvone, linalyl acetate, geraniol, lavandulyl acetate (**137**), 8-hydroxylinalool (**159a** or **159b**), neryl acetate	Beverages (herbal tea), perfume ingredients handmade soap Antifungal, antiinflammatory, antioxidative For anxiety disorders, calm, relaxant, eye strain
Lavandula hybrid (Lavandin oil)	Camphor, (+)-borneol, (−)-lavandulol, lavandulyl acetate	Flavor Antiseptic antimicrobial, expectorant For Alzheimer's and Parkinson's diseases, bronchitis, chest congestion, cold, flu, mental strengthen
Lavandula officinalis (Lavender oil)	α-Pinene, β-pinene, limonene, 1,8-cineole, linalool, linalyl acetate, lavandulol, lavandulyl acetate, citronellal	Flavor (skin lotion, soap perfume), beverages Antifungal For bronchitis, cold, coughs, cramps, dementia, eczema, flu, insomnia, menstrual disorders, pneumonia, ulcers

(continued)

Table 1 (continued)

Lens culinaris	Limonene, *o*-cymene, linalool, γ-terpinene, terpinen-4-ol, α-terpinyl acetate, *p*-cymen-7-ol, menthol	Foods Mucilaginous, laxative For constipation, intestinal affections, cleansing in foul, indolent ulcers
Lentinus edodes (Chinese mushroom)	*Fresh fruiting body*: camphene, β-pinene, limonene, linalool *Dried fruiting body*: β-pinene, sabinene, limonene, geraniol	Foods, beverages (herbal tea) Anticancer, hypocholesterolemia For cardiovascular, lower cholesterol, immunity booster
Leonurus japonicus (Chinese motherwort)	α-Pinene, camphene, fenchene, *p*-mentha-1,5,8-triene, menthone, *p*-menth-4-en-3-one, menthol, β-terpineol, perillyl acetate (**272**)	Beverages (herbal tea) For amenorrhea, blood circulation, blood stasis, dysmenorrhea, diuretics, dispelling edema, menstrual disturbance, postpartum hemorrhage, removing blood stasis
Ligustrum japonicum	α-Pinene, β-pinene, myrcene, limonene, borneol, α-terpineol	Beverages (herbal tea) Acaricidal for acaridae and pyroglyphid mites, antiinflammatory, antimicrobial, antiviral For dizziness, improving vision, menopause symptoms, soreness. tinnitus, vertigo
Lilium auratum (Golden-banded lily)	(*E*)-α-Ocimene, *p*-menthen-1-en-9-al (**221d**), linalool, (*Z*)-linalool oxide, (*E*)-linalool oxide, α-terpineol	Vegetable For mental and emotional health, reducing insomnia, healing burn, scars, wounds
Lilium lancifolium	Linalool	Vegetable, cosmetic For chronic bronchitis, skin care, up lighting, refreshing
Lilium leichtlinii	Linalool	Vegetable, cosmetic For chronic bronchitis, skin care, up lighting, refreshing
Lilium speciosum	Linalool	Vegetable, cosmetic For chronic bronchitis, skin care, up lighting, refreshing
Lindera strychnifolia (Lindera oil)	α-Pinene, camphene, β-pinene, myrcene, limonene, (*Z*)-β-ocimene, 1,8-cineole, *p*-cymene, terpinolene, fenchyl alcohol, fenchyl acetate, terpinen-4-ol, borneol, bornyl acetate	Beverages (herbal tea), aromatic Antifungal, antiphlogistic, antispasmodic, diuretic, tonic, vermicide For anodyne, abdominal, astringent, carminative, decongestant, diaphoretic, distension, dysuria, edema, enuresis febrifuge, menstrual cramps, scabies, stomach chills, stomachic

(continued)

Table 1 (continued)

Lindera umbellata	α-Pinene, Δ^3-carene, limonene, 1,8-cineole, linalool, geraniol, geranyl acetate, carvone	Beverages (herbal tea), cosmetics Antibacterial, anticancer, antifungal, antiinflammatory, antiobesity, expectorant For acute gastrointestinal, back pain, eczema, neuralgia, stiff neck
Liquidambar orientalis (Asiatic styrax)	α-Pinene, camphene, sabinene, β-pinene, α-phellandrene, α-terpinene, terpinolene, terpinen-4-yl acetate (**307b**), menthol, terpinen-4-ol, α-terpineol, (*E*)-piperitol, (*E*)-carveol, nerol, (*Z*)-myrtanol (**194b**), (*E*)-myrtenol, (*E*)-carveyl acetate	Oriental compounding flavor Bronchial catarrh
Liquidambar styraciflua (American styraciflua)	Tricyclene, α-thujene, α-pinene, camphene, sabinene, β-pinene, *p*-menth-1(7),8-diene, α-phellandrene, α-terpinene, *o*-cymene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, β-fenchyl alcohol, terpinolene, pinocarveol, (*E*)-verbenol, pinocarvone (**260**), borneol, terpinen-4-ol, *p*-cymen-9-ol, α-terpineol, (*Z*)-piperitol, verbenone, (*E*)-carveol, carvone	Natural perfumery incents. Antifungal, antiinflammatory, antimicrobial, antiseptic For alleviating pain
Litchi chinensis	Sabinene, linalool	Fruit Analgesic antibacterial, anticancer, antihyperlipidemic, antiplatelet, antitussive, antiviral, diuretic, hemostatic, hypoglycemic For cough, diabetes, epigastric, flatulence, hernia-like conditions, neuralgic pains, obesity, stomach ulcers, testicular swelling
Lonicera japonica	*p*-Cymene, (*Z*)-linalool oxide, (*E*)-linalool oxide, 1,8-cineole, linalool, (*Z*)-jasmine lactone (**132**), (*Z*)-jasmone (**133**), methyl *epi*-jasmonate, limonene, α-terpineol, geraniol	Beverages (herbal tea) For *Alveolar pyorrhea*, stomatitis, tonsillitis
Luffa cylindrica	Limonene, *p*-cymene, linalool, β-terpineol, camphor, cumin aldehyde (**71**), bornyl acetate	Vegetable Anthelmintic, antipyretic, tonic For emollient, galactagogic, jaundice, leprosy, lung complaints, nephritis, purgative, splenopathy, stomachic, syphilis, tumors

(continued)

Table 1 (continued)

Lycium chinense (Chinese wolfberry)	*Fruits/leaves*: limonene, terpinen-4-ol, menthol, linalool, geraniol, β-ionone, (*E*)-β-ionone-5,6-epoxide, dihydro-β-ionone, ionol	Fruit Antipyretic, diuretic, hypotensive, tonic
Lyophyllum shimeji	*p*-Cymen-9-ol	Foods
Magnolia obovata (=*M. hypoleuca*)	Tricyclene, α-pinene, camphene, β-pinene, myrcene, limonene, *m*-menth-1(7),8-diene (**202**), α-terpinene, *p*-cymene, linalool, 1,8-cineole, γ-terpinene, (4*Z*)-thujanol (**324**), terpinolene, fenchyl alcohol, pinocampheol [=(*E*)-3-pinanol] (**258c**), camphor, 3-methyl-camphenillol (**25**), terpinen-4-ol, α-terpineol, borneol, citronellol, neral, geraniol, geranial, bornyl acetate, geranyl acetate, methyl geranate	Food additives Antispasmodic, sedative For good sleep, detoxify, respiratory disorders
Mangifera indica (Mango oil)	α-Pinene, camphene, β-pinene, Δ^3-carene, limonene, sylvestrene, (**305**), β-phellandrene, α-phellandrene, β-ocimene, γ-terpinene, terpinolene, *p*-cymene, (*E*)-pinocarveol, terpinen-4-ol, cryptone, α-terpineol	Fruit Aphrodisiac, anticancer, antidiabetes, antiobesity, antioxidant, antistomachis, hypocholesterolemia, lower cholesterol, tonic For alkalize body, digestion, eye care, heart stroke, immunity system stimulate, removal of kidney stone, skin cleanser
Matricaria chamomilla (German chamomile oil)	α-Pinene, camphene, sabinene, yomogi alcohol, *p*-cymene, limonene, 1,8-cinecole (*E*)-β-ocimene, γ-terpinene, artemisia ketone (**5**), artemisia alcohol (**8**), linalool, terpinen-4-ol	Beverages (herbal tea), flavoring, cosmetics, soaps, mouthwashes Antiphlogistic antiinflammatory, antiseptic, antispasmodic, promoting relaxation For anxiety, chickenpox, diarrhea, eczema, hemorrhoids, indigestion, insomnia, mouth sores, stomach cramps, stomach ulcer
Matricaria recutita	α-Pinene, sabinene, myrcene, limonene, *p*-cymene, 1,8-cineole, (*E*)-β-ocimene, artemisia ketone, γ-terpinene, terpinen-4-ol, α-terpineol, carvone	Beverages (herb tea) Antimicrobial, antispasmodic

(continued)

Table 1 (continued)

Melaleuca quinquenervia	α-Pinene, α-fenchene, β-pinene, myrcene, limonene, α-terpinene, 1,8-cineole, γ-terpinene, (*E*)-β-ocimene, *p*-cymene, terpinolene, (*Z*)-linalool oxide (furanoid), (*E*)-linalool oxide (furanoid), linalool, terpinen-4-ol, *p*-menth-2-en-1-ol (**211b**), neral, terpinyl acetate, α-terpineol, geraniol	Beverages (herbal tea) Antispasmodic, antiseptic, antihelmintic, diaphoretic, expectorant
Melissa officinalis (Lemon balm)	α-Pinene, sabinene, β-pinene, myrcene, α-phellandrene, Δ^3-carene, *p*-cymene, *o*-cymene, limonene, β-phellandrene, γ-terpinene, terpinolene, linalool, (*E*)-thujene, camphor, *iso*-pulegol, citronellal, lavandulal (**135**), lavandulol (**136**), citronellol, neral, geranial, α-citral, neryl acetate, geraniol, geranyl acetate, geranic acid methyl ester (**96**), (*Z*)-carveol (**39b**), carvacrol	Foods, beverages, cosmetics Spice, fragrance Analgesic, antiaging, antibronchitis, antidepressant, antihypertensive, antimicrobial, antiviral, diaphoretic, insecticide, insect repellant, sedative, tranquilizer For headache
Mentha arvensis (Japanese mint oil)	Camphene, limonene, menthol (**183**), menthone (**189**), menthyl acetate (**184**), *neo*-menthol (**187**), piperitone, thymol	Food flavor (chewing gam, tobacco, cookies), crude drug Antifungal, antimicrobial
Mentha piperita (Peppermint oil)	α-Thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, Δ^3-carene, α-terpinene, *p*-cymene, 1,8-cineole, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, (*E*)-sabinene hydrate, terpinolene, (*Z*)-sabinene hydrate, linalool, (*Z*)-sabinol, menthone, *iso*-menthone, menthol, *iso*-menthol, *neoiso*-menthol (**188a**), myrtenol, pulegone, *iso*-pulegone, *neoiso*-menthyl acetate (**188b**), perillyl alcohol, thymol, menthyl acetate (**184**)	Beverages (peppermint tea, herbal tea, alcoholic drinks), spice, and for flavoring candy, ice cream, chewing gum and fruit preserves, toothpaste, shampoos, soaps Antispasmodic, carminative For anxiety, cold, flatulence, cold, indigestion

(continued)

Table 1 (continued)

Mentha piperita var. *vulgaris*	(−)-Menthol, menthyl acetate, menthone, menthofuran (**182a**)	Various food flavors Antiseptic, antiinfectious action For catarrhal congestion, clearing, colds, colic, coughs, diarrhea, digestive disorders, general fatigue, hay fever, headaches, impatience, indecision, indigestion, irritable bowel syndrome, jet lag, mental exhaustion, migraine, nausea, poor memory, sinus, stomach pains, vomiting
Mentha pulegium	α-Pinene, β-pinene, myrcene, limonene, 1,8-cineole, ocimene, *p*-cymene, *p*-mentha-1,3,8-triene (**204**), isophorone (**131**) *p*-menth-2-en-1-ol, (*E*)-sabinene hydrate, (*Z*)-sabinene hydrate, linalool, α-terpineol, (*Z*)-verbenol, menthone, neomenthol, menthofuran, menthol, menthyl acetate, borneol, *iso*-pulegone, (Z)-citral, α-terpinyl acetate, (*Z*)-piperitenone oxide, carveol, piperitone, carvone, thymol, linalyl butanoate (**170**), carvacrol, (*E*)-mentha-7(8)-dien-2-ol (**203a**), pulegone	Fragrance, flavoring, beverages (herbal tea) Antiseptic, antispasmodic, bowel disorders, carminative, diaphoretic, emmenagogue, flea-killing bath, insect repellent, pediculicide For mouth sores, mild CNS depressant, pneumonia, respiratory ailments, rubefacient, skin eruptions, stimulant, stomach pains, weakness
Mentha pulegium var. *eriantha* (Pennyroyal oil)	(+)-Pulegone, (−)-menthone, (+)-*iso*-menthone (**190**), piperitone, piperitenone (**275**), *iso*-piperitenone (**276**)	Soap flavor, source for menthol Antimicrobial, antioxidant
Mentha spicata (Spearmint oil)	α-Pinene, camphene, sabinene, β-pinene, myrcene, limonene, 1,8-cineole, (*Z*)-β-ocimene, (*Z*)-sabinene hydrate, linalool, (Z)-*p*-menth-2-en-1-ol, (*Z*)-limonene oxide, (*E*)-limonene oxide, δ-terpineol, terpinen-4-ol, borneol, dihydrocarveol (**37**), (*Z*)-dihydrocarvone (**43**), (*E*)-carveol (**38a**), (*Z*)-carveol (**39a**), pulegone, carvone, *iso*-bornyl acetate, *iso*-dihydrocarveyl acetate, (−)-carvone, menthone, pulegone, carvomenthone (**41**), dihydrocumin aldehyde (**72**)	Foods flavor (chewing gum), beverages (herbal remedy) For ailments, antiemetic, antioxidant, antispasmodic, carminative, diuretic, stimulant, stomachic For digestive disorders, fevers, headaches, restorative

(continued)

Table 1 (continued)

Mentha viridis	α-Pinene, myrcene, limonene, (*E*)-β-ocimene, α-terpineol, (*E*)-carveol, carvone, (*E*)-carveyl acetate (**38b**)	Beverages (herbal tea) Antiemetic, antimicrobial, antioxidant, antispasmodic, carminative, cytotoxicity, diaphoretic, diuretic, restorative, stimulant, stomachic For ailments, fevers, headaches, digestive disorder
Mercurialis leiocarpa	Myrtenol (**196**), myrtanal (**193**), geranial, myrtenal (**195**), citronellol, myrtanol (**194a** or **194b**)	Foods, beverages, dye
Momordica charantia	Linalool, (*E*)-linalool oxide, (*Z*)-linalool oxide, α-terpinolene, nerol, geraniol α-ionone, β-ionone, β-ionone-5,6-epoxide	Foods, beverages (herbal tea) Antidiabetes, antiinflammatory, antimicrobial, antioxidant, antitumor, antiviral, hypolipidemic For Hodgkin's disease, wound healing
Monarda citriodora (Lemon bee balm)	α-Pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, Δ^3-carene, α-terpinene, *p*-cymene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, linalool, α-terpineol, geraniol, citral, thymol, carvacrol	Beverages (herbal tea), fragrance Carminative, insect repellent For colds, coughs, fevers, and respiratory problems
Monostroma nitidum	β-Cyclocitral, α-ionone, β-ionone, β-ionone 5,6-epoxide	Foods, food additives, beverages Antiinflammatory, antioxidant, hypolipidemic
Morella rubra (=Myrica rubra)	Tricyclene, α-pinene, α-thujene, sabinene, myrcene, α-phellandrene, 1,4-cineole, 1,8-cineole, (Z)-β-ocimene, *iso*-limonene (**153**), linalool, (*E*)-linalool oxide, (*Z*)-linalool oxide, α-camphelenol (**27**), terpinen-4-ol, α-terpineol	Fruit Antiallergic, anticancer antiinflammatory, antimicrobial For cold, diarrhea, fever, joint pain, vomiting
Morinda citrifolia (Noni)	α-Pinene, sabinene, limonene, (*Z*)-β-ocimene, γ-terpinene, terpinolene, linalool, α-terpinolene	Foods, juice Analgesic, antiarthritic, anticancer, antidiabetes, antiemetic, antifungal, antiinflammatory, antimicrobial, antioxidant, antiparasitic, antiseptic, antituberculosis, antiviral, anxiolytic, sedative For immunity enhancing, memory enhancing, osteoporotic, otoscopy enhancer, wound healing

(continued)

Table 1 (continued)

Morus alba (Mulberry)	α-Pinene, α-thujene, camphene, Δ^3-carene limonene, *allo*-ocimene, (*Z*)-β-damascenone, geranyl acetone	Foods, beverages Analgesic, antibacterial, anticancer, antidiabetes, antihypertensive, antityrosinase, diaphoretic, gallstones dissolving, gastric acid neutralizing, hepatoprotective, sedative
Myristica fragrans (Nutmeg oil)	α-Pinene, β-pinene, sabinene, camphene, limonene, *p*-cymene, linalool, α-terpineol, borneol	Flavor food products, (cakes, cocky source, ketchup), spice Antiinflammatory, insecticide For cold, cough, digestion, fever, gastritis, nausea, pain, rheumatic, vomiting
Myroxylon balsamum var. *genuinum* (=*Toluifera balsamum, Myoxylon touiferu*) (Tolu balsam)	α-Pinene, β-pinene, linalool, carvone, geraniol, menthol, citronellal	Food aromatizer, beverages, flavoring (soft drinks, confectionaries, ice cream chewing gums), flavor for soap, cosmetics Antibacterial, antimicrobial, antiparasitic, antiseptic, bronchitis, catarrh, colds, expectorant, headache For asthma, flu, lung ailments, external wounds, rheumatism sores, sprains, tuberculosis, venereal diseases
Myroxylon pereirae (=*M. balsamum)* (Peru balsam)	α-Pinene, limonene, terpinolene	Food additives (gum, mucilage), food flavor, beverages (coffee, flavored beer, gin, liquor, tea, wine), cosmetics Antifungal, antimicrobial, antiseptic, expectorant For asthma, bronchitis, catarrh, chapped skin, cold, cough, eczema, external wounds, flu, poor circulation, rashes, rheumatism, sensitive skin, stress
Myrtus communis	α-Pinene, 1.8-cineole	Fragrance, infusion gargle
Myristica fragrans	α-Pinene, sabinene, β-pinene, myrcene, α-phellandrene, Δ^3-carene, *iso*-terpinolene (317), *o*-cymene, γ-terpinene, terpinolene, (*Z*)-4-thunanol (324), linalool, β-terpinyl acetate, (*E*)-*p*-menth-2-en-1-ol (211a), terpinen-4-ol, α-terpineol, (*Z*)-piperitol (263)	Spice Antibacterial, antiinflammatory, antioxidant

(continued)

Table 1 (continued)

Nandina domestica	Linalool, β-cyclocitral, *p*-cymen-9-ol	Beverages (herbal tea) Antipyretic, antitussive, tonic For asthma, flu, impotence, whooping cough, tired eyes
Narcissus jonquilla (Jonquil oil)	Linalool, α-terpineol	Flavoring agents, compound flavor Aphrodisiac, antispasmodic, narcotic
Nasturtium officinale (Water cress)	*Flower/stem*: α-pinene, β-pinene, myrcene, limonene, (*E*)-β-ocimene, α-terpinolene, α-terpineol, *p*-cymene-9-ol, pulegone *Leaf/ stem*: limonene, pulegone, *p*-cymen-9-ol, α-terpinolene	Vegetable Anticancer, antidiabetes, antimicrobial, antioxidant, antiscorbutic, antituberculosis, cardioprotective, dentalgia, depurative, diuretic, expectorant, hepatoprotective, hypoglycemic, purgative, stimulant, stomachic
Nelumbo nucifera (Lotus)	α-Pinene, 3,6,6-trimethyl-2-norpinene (**243**), sabinene, β-pinene, 1,4-cineole, terpinolene, *iso*-terpinolene (**313**), terpinen-4-ol, α-terpineol, (*E*)-*p*-menth-2-en-7-ol (**200**)	Foods (seeds), beverages (herbal tea) Analgesic, antimicrobial, antiinflammatory, antioxidant, gastrointestinal regulation, hypoglycemic, immunomodulatory
Nepeta cataria (Nepeta oil, Catnip oil)	α-Thujene, α-pinene, (*E*)-sabinene, β-pinene, Δ^3-carene, myrcene, limonene, γ-terpinene, linalool, 1,8-cineol, ascaridole (**9**), camphor, α-citral, δ-terpineol (**315**), iso-dihydronepetalactone (**227**), (*E*, *Z*)-nepetalactone (**228**), isopulegol, (*Z*,*E*)-nepetalactone (**229**), dihydronepetalactone (**226**), citronellal, citronellol, geranial, geraniol, neral, nerol, geranyl acetate, citronellyl acetate, thymol, nepetalic acid (**230**), nepetalic anhydride (**231**)	Foods, beverages, for making sauce, soup, herbal medicine Anticonvulsant, antiinflammatory, antimicrobial, antioxidant, antispasmodic, carminative, diuretic, insecticidal soporific, stimulant, sedative, tonic For anxiety, diarrhea. digestive, gastrointestinal and respiratory disorders such as asthma, bronchitis, colic, cough, headaches, hysteria, insanity, menstruating pain, nausea prevention, nervousness, relieve fevers, restlessness, sleeplessness, stress, sweating Felis attractant
Nepeta cataria	β-Pinene, ascaridole, limonene, citral, geraniol, citronellol, nerol, neo-nepetalactone (**225**), (*E*,*Z*)-nepetalactone (**228**), (*Z*, *E*)-nepetalactone (**229**)	Beverages (herbal tea) Antispasmodic, intestinal cramps, mild CNS stimulant, nervine For cancer, cold, colic, diarrhea, increase appetite, indigestion, menstruation disorders, sweating

(continued)

Table 1 (continued)

Nephelium lappaceum (Rambutan)	α-Pinene, limonene, (*E*)-geranyl acetone, β-damascenone	Fruit Antiallergic, antidiabetes, antioxidant, antiviral For coronary heart disease, diarrhea, dysentery, sperm health, strengthening bones
Ocimum basilicum (Basil oil)	α-Pinene, α-terpinene, γ-terpinene, *p*-cymene, 1,8-cineole, terpinolene, linalool, (*Z*)-linalool oxide. (*E*)-linalool oxide, (*E*)-sabinene hydrate, camphor, terpinen-4-ol, linalyl acetate (*E*)-*p*-menth-2-en-1-ol, bornyl acetate, carvacrol methyl ether, 3-methyl-camphenillol (**25**), (*Z*)-dihydrocarvone, terpinen-1-ol (**306**), menthol, (*E*)-pinocarveol, δ-terpineol, lavandulol, (*E*)-verbenol, α-terpinyl formate α-terpineol, borneol, dihydrocarveol, α-citral. verbenone, (*E*)-linalool oxide (pyranoid), (*Z*)-linalool oxide (pyranoid), (*Z*)-piperitol, citronellol, myrtenol, nerol, (*E*)-carveol, *p*-cymen-8-ol, geraniol, piperitenone, perillyl alcohol, cumin alcohol *Methyl chavicol type*: (methyl chavicol), linalool *Methyl cinnamate type*: (methyl cinnamate), linalool, methyl chavicol, 1,8-cineole *Camphor type*: camphor, camphene, limonene, borneol *Citral type*: citral, linalool, citronellol, citronellol *Thymol type*: thymol, carvacrol	Food flavor, spice, Italian, Indonesian, Vietnamese, Thai cuisine. Anthelmintic, antiaging, antibacterial, anticancer, antihyperglycemic, antiinflammatory, antifungal, antimicrobial, antioxidant, antiplasmodial, antitubercular, bronchitis, carminative, expectorant, insecticide, mild stimulant For cancer, cardiovascular diseases, constipation, coughs, diabetes diarrhea, headaches, kidney malfunction, mental health booster, rheumatoid arthritis,. warts, worms
Oenanthe javanica (Water dropwort)	α-Pinene, camphene, sabinene, β-pinene, myrcene, limonene, (*Z*)-β-ocimene, *p*-cymene, α-terpinene, γ-terpinene, α-terpinolene, linalool, bornyl acetate, carvacrol, borneol, γ-terpineol, linalool oxide, *p*-cymen-9-ol, terpinen-4-ol,	Vegetable, beverages (herbal tea), salad, seasoning in soups stews Analgesic, anticancer, anticoagulant, antifatigue, antiinflammatory, antinociceptive, antimutagenic, antioxidant, antithrombotic, antiviral, cardiovascular

(continued)

Table 1 (continued)

	thymol methyl ether, carvacrol methyl ether, geranyl acetone	protection, ethanol elimination, hepatoprotective, immune enhancement, insecticidal, hypoglycemic, neuroprotective For cough, depurative, discomfort, febrifuge, epidemic fever, headache, hematuria, influenza, jaundice, metrorrhagia, styptic
Olea europaea	Tricyclene, α-pinene, camphene, sabinene, β-pinene, myrcene, 2,3-dehydro-1,8-cineole (**53**), Δ^3-carene, *p*-cymene, limonene, 1,8-cineole, (*E*)-β-ocimene, γ-terpinene, (*Z*)-linalool oxide, linalool, (*Z*)-*p*-mentha-2,8-dien-1-ol (**209b**), camphor, menthone, umbellulone (**337**), menthol, terpinen-4-ol, α-terpineol, carvacrol methyl ether, *iso*-bornyl acetate, (*E*)-β-damascenone	Foods, beverages Antihypertensive, antioxidant, hypoglycemic, hypocholesterolemic, diuretic For asthma, bronchitis, cholagogue, diarrhea, emollient, febrifuge, gallstones, laxative, skin cleanser, urinary infections
Ophiocordyceps sinensis	*p*-Menth-4(8)-en-9-ol (**212**)	Beverages Antitumor, tonic For alcohol hepatitis, appetite, arthritis, bronchitis, chronic pain, coronary heart disease, longevity, diabetes, dizziness, erectile dysfunction, endurance, female aphrodisiac, general weakness, infertility, jaundice, malignant, liver and kidney diseases, longevity, malignant, prostate enlargement, rheumatism, sciatica and backache, sleeping patterns, tuberculosis
Origanum majorana (Origanum majorana leaf oil)	β-Phellandrene, *p*-cymene, β-thujene, linalool, (*E*)-2-menthen-1-ol (**211a**), (*Z*)-2-menthen-1-ol (**211b**), terpinen-4-ol, *p*-cymen-8-ol, α-terpineol, (*Z*)-piperitol (**263**), linalyl acetate, terpinyl acetate, dihydroumbellulone (**338**), terpinyl propanoate (**314**), geranyl acetone, carvacrol	Vegetables, curry powder, cooking food, fragrant Antimicrobial, antioxidant

(continued)

Table 1 (continued)

Origanum vulgare	Thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, limonene, α-terpinene, *p*-cymene, γ-terpinene, terpinolene, 1,8-cineol, linalool, β-ocimene, (*Z*)-linalool oxide, *p*-cymen-9-ol, borneol, thymol, carvacrol	Foods, spice Antibacterial, anticancer, antidiabetes mellitus, antifungal, antiinflammatory, antioxidant, carminative, digestive aid For bronchitis, cold sores, diarrhea, headache, menstrual pain, vomiting
Orthosiphon aristatus	β-Pinene, camphene, limonene, linalool, cyclocitral, carvone, camphor, borneol, bornyl acetate	Beverages Antidiabetes, antihepatitis, antihypertensive For edema, jaundice, kidney stone, rheumatism
Oryza sativa (Rice)	Limonene, *p*-cymene, linalool, geranyl acetone	Foods *Brown and germinated brown rice*: Anticancer, antidiabetes mellitus, antidiarrheal, antioxidant, reduction of cardiovascular risk
Osmanthus fragrans var. *aurantiacus* (Osmanthus oil)	Linalool, linalyl oxide, α-ionone, β-ionone, γ-decalactone, δ-dihydro-β-ionone (**126**)	Cosmetic fragrance Tea flavor attached
Paeonia lactiflora (Peony)	Perilla alcohol, thymol, carvacrol	Beverages (herbal tea) Atherosclerosis For chronic fatigue, syndrome (CFS), dysfunction, epilepsy, headache, liver cirrhosis, menstrual disorders, migraine, muscle cramps, nausea, neuralgia, sperms, upset stomach, viral hepatitis, whooping cough (pertussis)
Panax ginseng	β-Pinene, myrcene, *p*-cymene, β-phellandrene, limonene, α-terpineol	Foods, beverages, Kampo medicines. Aphrodisiac, antidepressant For improving both mental and performance, increasing resistance
Panax notoginseng	Camphene, β-pinene, limonene, β-phellandrene, α-terpinolene α-phellandrene	Beverages Antihypertensive For angina, intracranial hemorrhage, liver disorders, osteoarthritis, pain, stroke, swelling

(continued)

Table 1 (continued)

Panax quinquefolius (North American ginseng)	α-Pinene, camphene, β-pinene, limonene	Beverages Anti-Alzheimer's disease, anticancer, antidiabetes, antiinflammatory, antimicrobial, antiobesitic, antioxidant, anxiolytic, hypertrophy, improve cognitive, neuroprotective
Papaver somniferum (Poppy)	*Seeds*: α-pinene, *o*-cymene	Food additives, flavor, for cookies, bread, egg, grilled fishes Antibacterial, antiflu, antiinflammatory, anticancer, antiobesity, antitumor, detoxicant, expectorant, fungicide, herbicide, immunomodulatory, insecticide, nematocidal, pesticide, photo sensitizer, sedative
Pastinata sativa (Parsnip)	Terpinolene	Vegetable Antioxidant For psoriasis, vitiligo
Paxillus involutus	1,8-Cineole	Foods (cooked)
Pelargonium denticulatum	Geraniol, citronellol	Antibacterial, antifungal
Pelargonium graveolens	α-Pinene, myrcene, *p*-cymene, limonene, γ-terpinene, linalool, (*Z*)-linalool oxide, (*E*)-rose oxide (**291**), (*E*)-linalool oxide, (*Z*)-rose oxide (**292**), *neoiso*-pulegol (**284**), menthone, *iso*-menthone (**190**), *iso*-menthol (**186a**), α-terpineol, citronellol, neral, geraniol, neryl formate (**235**), citronellic acid (**56**), geranic acid methyl ester, 8-hydroxy-*neo*-menthol (**185**), citronellyl acetate (**59**), citronellyl propionate (**60b**), citronellyl isobutanoate (**62**), citronellyl butanoate (**61**), citronellyl 2-methylbutanoate (**63**), citronellyl pentanoate (**64**), citronellyl heptanoate (**65**), lavandulyl 2-methylbutanoate (**139**), lavandulyl tiglate (**140**),	Beverages, cosmetic, soap, fragrance, lotions Antianxiety, antiaging, antibacterial, antifungal for food spoilage pathogens, antiinflammatory, antioxidant, antiviral For anxiety, aiding, depression, burns, calming nerves, digestive, ulcers, abscesses, cold sores, creating a relaxing, uplifting feeling, nerve pain relief, premenstrual tension, respiratory ailments, sore throats, ulcers, abscesses, wounds

(continued)

Table 1 (continued)

	geranyl formate (**98**), geranyl propanoate (**100**), geranyl isobutanoate (**102**), geranyl butanoate (**101**), geranyl 2-methylbutanoate (**105**), geranyl pentanoate (**103**), geranyl tiglate (**106**), geranyl hexanoate (**107**), geranyl heptanoate (**108**), geranyl octanoate (**109**)	
Pelargonium odoratissimum	Thujene, α-pinene, sabinene, β-pinene, myrcene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, ocimene, γ-terpinene, linalool, fenchone, fenchyl alcohol, camphor, menthone, *iso*-menthone (**190**), *iso*-borneol, terpinen-4-ol, α-terpineol, citronellol, (*E*)-carveol, (*Z*)-carveol, piperitone	Beverages, apple aroma Antiseptic, astringent, tonic For aromatherapy, debility, gastroenteritis, hemorrhage, injuries, mouthwash, neuralgia, ointment, skin complaints, throat infections
Pelargonium radula (Geranium oil)	Geraniol, citronellol, *iso*-menthone, citronellol, citronellyl formate (**60a**), geranyl formate (**98**), geranyl acetate (**97**)	Food flavoring (jellies), food additives, beverages (herbal tea) Antibacterial, antifungal
Pelargonium roseum	α-Pinene, linalool, geraniol, menthone, citronellol, citronellyl formate, geranyl acetone (**111**)	Analgesic, antibacterial, antifungal. antiinflammatory, antiseptic, astringent
Perilla frutescens (=*P. frutescens* var. *frutescens*, *P. frutescens* var. *japonica*) (Egoma)	*Leaves:* α-pinene, β-pinene, limonene, *p*-cymene, linalool, linalyl acetate, linalyl senecioate (**173**), geraniol, elsholtzia ketone (**87b**), naginata ketone (**224**), perilla ketone (**273**), *iso*-egomaketone (**87a**) *Seeds:* (Himalaya): 1,8-cineole, γ-terpinene, linalool, perilla ketone, *iso*-perilla ketone (**290**)	Vegetable, food additives, flavor Anticancer, antiinflammatory, antimicrobial, antispasmodic, inducing sweating For allergies, asthma, heart diseases, colitis/Crohn's disease, heart diseases, nausea, sunstroke
Perilla frutescens var. *crispa* f. *purpurea* (Akajiso)	Linalool, perillaldehyde, shisool	Vegetable, beverages, food additive, flavor, species Leaf: food as Japanese cuisines (Tempura, Sushi)
Perilla frutescens var. *crispa* f. *viridis* (Aojiso) (*Perilla* oil)	α-Pinene, camphene, β-pinene, myrcene, limonene, *p*-cymene, (*E*)-linalool oxide, (*Z*)-linalool oxide, linalool, (−)-menthol, α-terpineol, carvone, (−)-perillaldehyde, (*E*)-carveol (**38a**), (Z)-carveol (**39a**), (*E*)-	Vegetable, food additives Flavor *Leaves*: food as Japanese cuisines (Tempura, Sushi) Analgesic, antiallergenic, antibacterial, antibiotic, anticorrosion, antifungal,

(continued)

Table 1 (continued)

	shisool (**303**), (*Z*)-shisool (**304**), (−)-perilla alcohol (**271**), perilla ketone, *iso*-egomaketone (**87a**), shisofuran (**302**), (*Z*)-jasmone, thymoquinone (**335**), carvone	antiinflammatory, antioxidant, antipyretic, antitumor, antitussive, apoptosis inducing, insecticidal, sedative, stomachic For asthma, constipation, back and muscle pain, cold, intestinal disorders
Perilla frutescent var. *crispa* f. *crispa* (leaf) (Chirimenjiso)	(*E*)-Linalool oxide, (*Z*)-linalool oxide, linalool, pinanol, α-terpineol, perillaldehyde, 10-pinanol [=(*E*)-myrtanol)] (**194a**), perilla alcohol, geraniol	Vegetable, beverages, food additives, flavor, species *Leaves*: food as Japanese cuisines (Tempura, Sushi) Antiinflammatory, antimicrobial
Persea americana (Avocado oil)	α-Pinene, camphene, sabinene, β-pinene, myrcene, limonene, β-ocimene, α-phellandrene, α-terpinene, 1,8-cineole, γ-terpinene, carvone, neryl acetate	Foods, food flavor Antihypertensive, antioxidant, hypocholesterolemia For aphrodisiac, coughs, diarrhea, dysentery, gout, hair growth, liver disorders, smooth skin
Persicaria tinctoria	Limonene	Vegetable, beverages, cookies Antidote, antiinflammatory, antiphlogistic, antipyretic, depurative For epidemic proctitis, erysipelas, freckles, infantile convulsions and high febrile conditions of children, mumps, pimples, thrush
Petasites japonicus	α-Pinene, camphene, β-pinene, myrcene, limonene, α-phellandrene, Δ^3-carene, p-cymene, perillaldehyde, linalool, γ-terpinene, *p*-menth-1-en-9-ol, thymol methyl ether, linalyl propanoate (**169**)	Vegetable, beverages (herbal tea) Antitussive, tonsillitis For headache, discomfort of gastrointestinal system, menstrual wellness
Petroselinum crispum (Parsley)	α-Thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, Δ^3-carene, α-phellandrene, α-terpinene, limonene, β-phellandrene, (*E*)-β-ocimene, (*Z*)-β-ocimene, γ-terpinene, *p*-cymene, α-terpinolene, *p*-mentha-1,3,8-triene, α-terpineol, *p*-cymen-8-ol, cryptone, α-campholenal (**28**), (*E*)-pinocarveol (**258a**), pinocarvone, myrtenal, myrtenyl acetate (**197**)	Vegetable, beverage (herbal tea), tonic Antihypertensive, antimicrobial, antiseptic, antispasmodic, diuretic, stimulant For abortifacient, amenorrhea, anemia, diabetes, dysmenorrhea, gastrointestinal disorders, halitosis, insect bite, kidney stones

(continued)

Table 1 (continued)

Peucedanum japonicum	α-Thujene (**329**), α-pinene, α-fenchene, camphene, β-pinene, myrcene, Δ^3-carene, α-phellandrene, α-terpinene, *p*-cymene, limonene, β-phellandrene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene, *allo*-ocimene, (*E*)-limonene oxide, (*E*)-pinocarveol, lavandulol, terpinen-4-ol, verbenol, α-terpineol, β-citronellol, pinocarvone, cryptone, piperitone, thymol, nerol, campholenal (**28**), neral, geranial, linalyl acetate, isobornyl acetate, bornyl acetate, sabinyl acetate, α-fenchyl acetate, citronellyl acetate, thymol methyl ether, carvacrol methyl ether	Vegetable juice, beverages Antiasthmatic, antiinflammatory For allergic, lung inflammation, cold, cough, headache, liver, lung, kidney, spleen stomach meridians, rental disorder, typhoid fever
Phaseolus vulgaris	α-Pinene, Δ^3-carene, limonene, geranyl acetone	Vegetable For diabetes, obesity, weight loss
Phellodendron amurense	*Leaves*: α-pinene, camphene, sabinene, myrcene, α-phellandrene, Δ^3-carene, limonene, (Z)-β-ocimene, γ-terpinene, limonene oxide, camphor, piperitone oxide *Seeds*: tricyclene, α-pinene, myrcene, β-citronellol, geranyl acetone	Beverages (herbal tea) Antiinflammatory, bitter stomach medicine For anemia, anorexia, convergence, diarrhea, gastric spasm, pneumonia, pulmonary tuberculosis, sterilization
Phyllostachys heterocycla cv. *pubescens*	Menthone, geranyl acetone, α-ionone, damascenone, β-ionone	Food, beverages Antimicrobial
Phyllostachys nigra	Citronellyl isovalerate *Fermented:* limonene, linalool	Foods (young shoots), beverages (herbal tea). Antiemetic, antipyretic, antitussive, astringent, depurative, diuretic, expectorant, hemostasis, sedative, tonic For asthma, cough, fevers, nose bleeds, phlegm, pneumonia, rabies, styptic, vomiting

(continued)

Table 1 (continued)

Physalis peruviana (Cape gooseberry)	α-Pinene, camphene, verbenene, dehydrosabinene (**293**), myrcene, α-terpinene, limonene, 1,8-cineole, (*E*)-β-ocimene, *p*-cymene, α-terpinolene, rose oxide, linalool oxide, isopulegol, α-fenchyl alcohol, terpinen-4-ol, β-cyclocitral, (*Z*)-citral, limonene 4-ol (=mentha-1,8-dien-4-ol) (**157**), α-terpineol, borneol, myrtenal, verbenone, geranial, β-citronellol, nopol (**242**), (*Z*)-*p*-menta-1(7),8-dien-2-ol (**203b**) geraniol, *p*-cymen-9-ol, geranyl acetone, (*Z*)-myrtanol (**194b**), carvacrol, neric acid (**233**), dihydroactinidiolide (**3**)	Fruit, jam, pies, puddings, chutneys, salad, cocktail Anticancer, antimycobacterial, antioxidant, antipyretic, diuretic, eye sight improving, immunomodulatory, liver and kidney against fibrosis protecting For arthritis pain, asthma, dermatitis, hepatitis, malaria, leukemia, rheumatism
Pimenta dioica (Allspice)	α-Pinene, sabinene, myrcene, limonene, α-phellandrene, Δ^3-carene, α-terpinene, *p*-cymene, 1,8-cineole, (*Z*)-β-ocimene, γ-terpinene, terpinolene, linalool, *p*-menth-2-en-1-ol, terpinen-4-ol, *p*-cymen-8-ol, α-terpineol, α-phellandrene epoxide, (*E*)-piperitol	Foods, beverages, flavor, perfumery, soup. Mulled wine, sider Anesthetic, antiseptic, fungicide, pesticide For ailments, flatulence, muscle pain, upset stomachic
Pimenta officinalis (Pimenta oil, allspice oil)	α-Phellandrene, 1,8-cineole	Food flavor (meats, source, pickles, cookies), spice Anticancer, antidiabetes, antifungal, antimicrobial, antioxidant, carminative, nematocidal
Pimenta racemosa (Bay oil)	α-Pinene, β-pinene myrcene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, α-phellandrene, (*E*)-β-ocimene, γ-terpinene, terpinolene, linalool, terpnen-4-ol, α-terpineol, citral	Food flavor, table source, cosmetic water flavor, hair cosmetic product Acaricidal, antifungal antiinflammatory, antimicrobial, antioxidant
Pine nut from *Pinus cembroides* *P. geradiana* *P. koraiensis* *P. monophylla* *P. odules* *P. pinea*	α-Pinene, camphene, β-pinene, Δ^3-carene, limonene, *p*-menth-1-en-8-ol, terpinen-4-ol, verbenone, borneol, bornyl acetate	Beverages Antitussive, insulation, sedative, brain and stomachic activation
Pinellia ternata	*o*-Cymene, limonene, linalool, menthone, borneol, menthol, terpinen-4-ol, citronellol, pulegone, *iso*-pulegone, (*E*)-*p*-menth-2-en-7-ol, cumin alcohol	Beverages (herbal tea) For sore throat

(continued)

Table 1 (continued)

Pinus densiflora (Turpentine oil)	α-Pinene, β-pinene, camphene, sabinene, myrcene, α-ocimene, α-terpinene, terpinolene α-terpineol	Foods (seed), production of pinene For bladder, kidney, skin complaints, cough, cold, influenza, rheumatic, rubefacient
Pinus palustris (Pine oil)	Terpineol, α-fenchyl alcohol, borneol	Flavor Antiseptic, bactericides, disinfectant, diuretic, vermifuge For cough, chronic, cold, colic, diarrhea, worms
Piper betel	*Betel leaf oil*: *p*-cymene, 1,8-cineole, (Z)-sabinene hydrate (**297**), carvacrol	Foods, beverages, flavor. Anticancer, antimalarial, antimicrobial, expectorant, hypocholesterolemic For asthma, diabetes, good stomachic, healing wounds, luxury, oral health
Piper nigrum	*Pepper oil*: α-thujene, α-pinene, sabinene, β-pinene, myrcene, Δ^3-carene, limonene, α-phellandrene, β-phellandrene, γ-terpinene, cryptone, terpinolene, α-terpinene, *p*-cymene, dihydrocarveol (**37**)	Spice (black and white pepper), food additives (sausage, soup, source, cans
Piptoporus betulinus (Polypore)	Limonene	Foods, beverages (herbal tea) Antiseptic, antifungal, tonic for the immune system For parasitic worms, wound healing
Pittosporum tobira	α-Pinene, β-pinene, myrcene, limonene, (*E*)-β-ocimene	Beverages (herbal tea) Anticancer, antifungal, antiinflammatory, antioxidant, antiproliferative
Pleurotus ostreatus (Oyster mushroom)	α-Pinene, *p*-cymene, linalool, (*E*)-linalool oxide	Foods Anticancer, atherosclerosis, antioxidant, hypolipidemic For diabetes, immune system booster, respiratory tract infection
Pogostemon cablin (Patchouli oil)	α-Pinene, β-pinene, camphene, limonene, myrtenol, α-terpineol, camphor, cumin aldehyde	Breath fresheners, incent agent, soap perfume
Polianthes tuberosa (Tuberose oil)	Geraniol, nerol, geranyl acetate, neryl acetate	Luxury compounded fragrance

(continued)

Table 1 (continued)

Polygonatum odoratum	Citronellol	Adaptogenic, allergic, antibacterial, antimicrobial, antioxidant, antiperiodic, antitussive, cardiotonic, demulcent, diuretic, energizer, hypoglycemic, ophthalmic, resolvent, sedative, tonic For dry throat, dry coughs, coronary heart disease, skin toner
Polygonum minus (Kesum oil)	α-Thujene, α-pinene, limonene, β-ocimene, myrtenol, myrtenol, borneol, geraniol, geranyl acetone	Vegetable Aggregation, antifungal, antiinflammatory antimicrobial, antioxidant, antiplatelet, antiproliferative, antiulcer, cytotoxicity, cognitive and digestive enhancing, gastroprotective, genotoxicity, immunomodulatory For body aches, digestive disorders, stomachic
Polygonum hydropiper (Water pepper)	α-Pinene, myrcene, dihydro-α-ionone (**121**)	Vegetable, spice, beverages (tea) For diarrhea, dyspepsia, itching skin, menstrual bleeding, hemorrhoids, poultice for swollen and inflamed areas
Porphyra tenera	Piperitone oxide (**281b**), β-cyclocitral, α-ionone, neryl acetone (**240**), β-ionone	Foods, food additives Antiinflammatory, antioxidant
Prunella vulgaris	Limonene, linalool, (*E*)-linalyl oxide, (*Z*)-linalyl oxide, β-cyclocitral, geranyl acetone	Herbal medicine. Antiinflammatory
Prunella vulgaris var. *lilacina*	Limonene, camphor, linalool, linalool oxide, β-cyclocitral, geranyl acetone	Antiallergic, anticancer, antiinflammatory, antimicrobial, antioxidant, antiviral, diuretic, tonic, astringent For beriberi, cystitis, hemostasis, sore throat, stomatitis, tonsillitis
Prunus amygdalus var. *amara* (Bitter almond oil)	*	Food flavor (cakes, wine)
Prunus armeniaca (Apricot oil)	Limonene, (*E*)-β-ocimene, linalool, dihydro-β-ionone (**125**), dihydroactinidiolide, damascone, β-ionone, (*E*)-rose oxide (**291**)	Fruit, flavor, crude drugs

(continued)

Table 1 (continued)

Prunus mume (Ume oil)	*Fruit: p*-cymene, (*E*)-linalool oxide, (*Z*)-linalool oxide	Fruit, food flavor (fruit oil)
Prunus persica (Peach oil)	Linalool	Fruit Anticancer, antidiabetes, antiinflammatory, antioxidant, hypocholesterolemic For bone and kidney disorders, cardiovascular, skin health, eye vision
Pseudocydonia sinensis	Linalool, terpinen-4-ol, α-terpineol, neral, geranial	Beverages, alcoholic beverage Anticancer, antimicrobial, antioxidant, diuretic, expectorant For cough, diarrhea, pharyngitis
Pseudocydonia sinensis (Chinese quince) (=*Cydonia sinensis*)	Limonene, (*E*)-theaspirane (**319**), (*Z*)-theaspirane (**320**), carvone, (*E*)-linalyl oxide, (*Z*)-linalyl oxide	Candied fruit, fruit liquor Antiinflammatory, antioxidant For cough, diuretic
Psidium guajava	*White fruit* (*head space*): α-pinene, 1,8-cinenole, borneol, cumin alcohol *Essential oils (leaf/fruits):* α-pinene, β-pinene, Δ^2-carene (**30**), α-phellandrene, α-terpinene, *p*-cymene, limonene, 1,8-cineol, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene, *allo*-ocimene, α-terpineol, carvone	Fruit Antibacterial, anticough, antidiabetes, antihelmintic, antimicrobial, antioxidant, antiproliferation, antispasmodic, cooling, laxative, tonic For diarrhea in children, pulmonary disorders, relief of cough, ulcers, wounds
Psidium guajava	*Fruits/leaves:* camphene, sabinene, limonene, *(Z)*-β-ocimene, 1,8-cineole, α-terpineol, citral, β-ionone	Fruit Antiatopic, antidiabetes, antihypertension, dermatitis For cerebral infection, intestinal regulation lightening, stomachic
Pteridium aquilinum	α-Thujene, α-pinene, sabinene, β-pinene, myrcene, limonene, α-terpinene, 1,8-cineole, (*Z*)-β-ocimene, *allo*-ocimene, γ-terpinene, terpinolene, fenchone, neral, geranial, *iso*-artemisia ketone (**6a**), nerol, geraniol	Foods, source of starch, herbal medicine Antimicrobial, antioxidant, antiparasitic, jaundices

(continued)

Table 1 (continued)

Pueraria montana var. *lobata*	α-Thujene, α-pinene, sabinene, β-pinene, myrcene, γ-terpinene, limonene, α-terpinene 1,8-cineole, (*Z*)-β-ocimene, *allo*-ocimene, γ-terpinene, linalool, fenchone, neral, geranial, *iso*-artemisia ketone (**6a**)	Foods, source of starch, beverages (herbal tea) Antihypertension For alcoholism, angina, angioplasty, cold, diabetic, hay fever, headache, flu, heart failure, dizziness, ischemic stroke, nephropathy, retinopathy, upset stomach, sinus infections, swine flu, vomiting
Punica granatum	α-Thujene, α-pinene, sabinene, β-pinene, camphene, limonene, Δ^3-carene, α-phellandrene, γ-terpinene, *p*-cymene, β-phellandrene, camphor, α-terpineol	Foods, beverages (juice), food seasoning, cosmetics Anticancer, antidiabetes, antihelmintic, antiinflammatory, antimicrobial, antiobesity, antioxidant, antiswelling, diarrhea, dysentery, stomachache, suppression for melanin
Pyropia tenera	Limonene, α-terpineol, α-ionone, β-ionone, dihydroactinidiolide	Foods Anticancer, antiinflammatory For immune system booster
Raphanus sativus (Red radish)	Δ^3-Carene	Foods, vegetable For appetite, bronchitis, chronic diarrhea, colds, cough, fever, intestinal, liver and stomach disorders, gallstones, high cholesterol
Raphanus sativus var. *longipinnatus*	Camphene, myrcene, α-terpinene, limonene, 1,8-cineole, *m*-cymene (**76**), terpinene-4-ol, *p*-menth-6-en-3-one (**221e**), piperitone, carvone, camphor, *neoiso*-menthol, dihydrocarveol, α-terpineol, menthol, menthyl acetate, nerol, bornyl acetate, carvacrol, geranyl acetone	Foods, vegetable Antidiabetes, antimicrobial, antiobesity
Rheum rhaponticum (Rhubarb, garden rhubarb)	Limonene, linalool, β-ionone, α-terpineol, (*E*)-geranyl acetone	Foods, beverages, syrups, liqueurs, jam, compote, juice, ingredients in ice cream, yogurt, candies Antidiarrheal, laxative
Rosa damascena f. *trigintipetala* (Rose oil)	Linalool, (−)-citronellol, geraniol, nerol, geraniol, (*E*- or *Z*-rose oxide (**291** or **292**)	Food flavor, beverages (rose water) Antispasmodic, relaxant, stomachic

(continued)

Table 1 (continued)

Rosa rugosa (Hamanasu oil)	α-Pinene, β-pinene, myrcene, limonene, 3-carene, (*E*)-β-ocimene, linalool, (*E*)-rose oxide (**291**), citronellal, geraniol, citronellol, α-citral, nerol, geranyl acetate, neryl acetate, citronellyl acetate	Foods, perfume, cosmetics, spices
Rosmarinus officinalis (Rosemary oil)	α-Pinene, camphene, β-pinene, limonene, thyuja-2,4(10)-diene (**323**), α-phellandrene, α-terpinene, *p*-cymene, 1,8-cineole, γ-terpinene, *p*-mentha-2,4(8)-diene (**208**), terpinolene, linalool, chrysanthenone, camphor, pinocarvone, menthol, borneol, terpinen-4-ol, α-terpineol, verbenone, bornyl acetate	Flavoring food, cosmetic, perfume, spice, spray, crude drug, beverages (herb tea) Antiinflammatory, antimicrobial, antioxidant, antispasmodic, carminative, GI irritant For hair growth, hepatic, intestinal renal, respiratory affections, skin health
Rubus idaeus	α-Pinene, camphene, sabinene, β-pinene, myrcene, limonene, Δ^3-carene, *p*-cymene α-phellandrene, (*E*)-β-ocimene, terpinolene, (*Z*)-β-ocimene, 1,8-cineole, α-terpinene, β-phellandrene, γ-terpinene, linalool, (*E*)-linalool oxide (furanoid), (*E*)-linalool oxide (pyranoid), (*Z*)-linalool oxide (pyranoid), linalyl acetate, terpinen-4-ol, (*Z*)-piperitol (**263**), (*Z*)-sabinol (**300**), α-terpineol, camphor, menthone, menthol, menthyl acetate, myrtenol, *neo-allo*-ocimene (**244**), *p*-cymen-9-ol, 8-hydroxylinalool, neral, nerol, cyclocitral, verbenone, dihydrolinalool, geranial, geraniol, *iso*-piperitenone, (*Z*)-jasmone, theaspirane A (**319**), theaspirane B (**320**), theaspirane I, theaspirane II, dihydroactinidiolide, α-ionone, β-ionone, damascenone, dihydro-α-ionone (**121**), dihydro-β-ionone (**125**)	Foods, fruit Anticancer, antidiabetes, antifungal, antihypertension, antiinflammatory, antiobesity, antioxidant For Alzheimer's and Parkinson's diseases, chronic diseases, digestion, eye and heart health

(continued)

Table 1 (continued)

Russula cyanoxantha	α-Pinene, 1,4-cineole, limonene, 1,8-cineole, linalool, α-terpineol, geranyl acetone	Foods
Ruta graveolens	Geijerene (**93**), geyrene (**115**), piperitenone (**275**), (*E*)-piperitenone oxide (**285**), (*Z*)-piperitone oxide (**289**), (*Z*)-*iso*-pulegone (**268**)	Beverages Antidiabetes, antidiarrhetic, antifebrile, antimicrobial, antiinflammatory, antiparasitic, antirheumatic, antiulcer, healing, vermicide For cramps, earache, headache, diabetes, menstrual disorders
Salvia officinalis (Sage oil)	α-Thujene, tricyclene, α-pinene, camphene, sabinene, β-pinene, α-phellandrene, α-terpinene, *p*-cymene, limonene, 1,8-cineol, γ-terpinene, terpinolene, camphor, terpinen-4-ol, α-terpineol, borneol, bornyl acetate, α-thujone (**331a**), β-thujone (**331b**)	Foods, food flavor (sausage, pickles, soup, canes, vine), beverages, crude drugs, spice Antiinflammatory, antioxidant, anthelmintic, antisecretary, astringent, diuretic, emmenagogue, insect repellent, nitric oxide production and NF-kB transcription factor inhibitory, strengthen bones and immune system For asthma, gastroenteritis, cognitive disorder, digestion, mouthwashes, wound healing
Salvia sclarea (Clary sage oil)	α-Pinene, sabinene, β-pinene, myrcene, limonene, 1,8-cineol, (*Z*)-β-ocimene, linalool, (*E*)-β-ocimene, *allo*-ocimene, α-terpinene, γ-terpinene, terpinolene, α-phellandrene, β-phellandrene, (*E*)-linalool oxide (furanoid), (*Z*)-linalool oxide (furanoid), linalyl acetate, camphor, *p*-cymen-8-ol, *iso*-borneol, linalyl formate, terpinen-4-ol, neral, lavandulol, geranyl formate, geranial, geranyl acetate, α-terpineol, α-terpinyl acetate, neryl acetate, damascenone, nerol, nerol oxide, cumenol (**68b**), geraniol, thymol, carvacrol, carvacrol methyl ether	Food flavor beverages (herbal tea) Antibacterial, antidepressant, antiinflammatory, antispasmodic, tonic For indigestion, circulation, stress relief, pain, and premenstrual problems

(continued)

Table 1 (continued)

Sambucus nigra (Elderberry)	Limonene, linalool, bornyl acetate, (*E*)-β-damascenone, carvacrol	Foods, jam, marmalade, pies, beverages (wine, liqueurs, juice, tea) Antiinflammatory, diuretic, diaphoretic, laxative For flu, stimulating immune system, well-being
Santalum album (Sandalwood oil)	Camphene, α-terpinene	Food products, macadamia nut, bush food preparation, oriental flavor, cosmetics, soap flavor, aroma retaining agents For cold, liver, digestive gallbladder, hemorrhoids, muscle problems, mental disorders, scabies, urinary tract infections
Saposhnikovia divaricata	α-Pinene, β-pinene, myrcene, *o*-cymene	Vegetable Analgesic, anticancer, antiinflammatory, antioxidative, antiproliferative, immunoregulatory
Sasa veitchii	Linalool, α-terpineol, geranyl acetone	Beverages (herbal tea) Antihypertension, anticancer, antiinflammatory, antimicrobial, cardiotonic For constipation, diabetes mellitus, stomachic, ulcer
Sassafras albidum (=*Sassafras variifolium*) (Sassafras oil)	α-Pinene, myrcene, α-phellandrene, β-phellandrene, linalool, camphor, citral, geraniol, geraniol esters	Foods, food additives, beverages (herbal tea), perfume Analgesic, anticancer, antifungal, antimicrobial, antipyretic, antiseptic, carminative, diaphoresis For acne, bronchitis, dentifrice, diaphoretic, dysentery, menstrual disorders, kidney problem, rheumatism, rubefacient, skin sores, sudorific, swelling, trauma, urinary disorders
Satureja hortensis	α-Pinene, myrcene, camphene, β-pinene, myrcene, α-phellandrene,, α-terpinene, *p*-cymene, *o*-cymene, γ-terpinene, thymol, carvacrol	Flavor, garnish, beverages (herbal tea) Sedative For cramps, diarrhea
Satureja montana (Winter savory)	p-Cymene, γ-terpinene, borneol, geraniol, thymol, carvacrol, thymol methyl ether, carvacrol methyl ether	Flavoring for cooked foods, garnish for salads, beverages (herbal tea), liquors

(continued)

Table 1 (continued)

Saxifraga stolonifera	α-Pinene, cumene, *p*-cymene, terpinolene, linalool, α-terpineol, geraniol geranyl acetate, citronellol	Vegetable Antiaging, anticancer, antioxidant
Sechium edule (=*Chayota edulis*)	Limonene, *p*-cymene	Vegetable Antidiabetes mellitus, antineoplastic, antioxidant
Sesamum indicum	α-Pinene, myrcene, linalool, carvomenthone, geraniol, geranial, neral, fenchyl alcohol, *iso*-pulegol, thymol	Foods Antiaging, antihyperlipidemic, antihypertension For AIDS-related wasting, bowel obstruction, burns, cough, diabetes, gingivitis, lower blood pressure, serum lipid
Solanum lycopersicum (=Lycopersicon esculentum)	*Fresh tomato head space*: β-phellandrene, linalool, geranial, neral, β-cyclocitral, 5,6-epoxy-β-ionone *Leaves (essential oils):* α-thujene, α-pinene, Δ^2-carene, β-pinene, myrcene, limonene, α-phellandrene, α-terpinene, *p*-cymene, γ-terpinene, (*Z*)-β-ocimene, (*E*)-β-ocimene, β-phellandrene, terpinolene, 1,8-dineole, Δ^2-carene epoxide (**32**), limonene epoxide, linalool, neral, geranial, geraniol *Whole, pulp, fluid*: linalool, nerol, geranial, β-cyclocitral, geranyl acetone	Foods, processed foods (ketchup, paste), vegetable, beverages Anticancer, antidiabetes, antiosteoporosis, antioxidant, immunity booster, detoxification For cardiovascular disease, fatigue, liver health, sleepiness, strengthening bone Food flavor
Solanum melongena (Eggplant, aubergine)	Δ^3-Carene, linalool	Vegetable For asthenia, bronchitis, cholera, diabetes, dysentery, dysuria, hemorrhoids, otitis, skin infections, toothache
Solanum tuberosum (Potato)	Tricyclene, α-pinene, sabinene, limonene, myrcene, linalool, 1,8-cineole	Vegetable (salad, bug, soup, gratin), source of starch, alcoholic beverages (vodka, poitin, or akvavit)
Spinacia oleracea (Spinach)	β-Cyclocitral, geranyl acetone, β-ionone epoxide	Vegetable For stomach and intestinal complaints, fatigue, stimulating growth in children, recovery from illness

(continued)

Table 1 (continued)

Stevia rebaudiana	α-Pinene, sabinene, β-pinene, limonene, (*E*)-β-ocimene, bornylene (**22**), linalool, (*Z*)-linalool oxide, (*E*)-linalool oxide, terpnen-4-ol, α-terpineol, myrtenol, *p*-menth-1-en-9-ol (**206**), β-cyclocitral, neral, nerol, geraniol, carvacrol	Food additive (sweetener). Antimicrobial, contraceptive, hypoglycemic, hypotension regulation
Suillus bellinii	α-Pinene, β-pinene, 1,4-cineole, limonene, 1,8-cineole, linalool, menthol, geranyl acetone, β-ionone	Foods
Suillus granulatus	α-Pinene, β-pinene, 1,4-cineole, limonene, 1,8-cineole, linalool, menthol, α-terpineol, geranyl acetone, β-ionone	Foods
Suillus grevillei	Limonene, geranyl acetone	Foods
Suillus luteus	α-Pinene, β-pinene, 1,4-cineole, limonene, linalool, menthol, α-terpineol, geranyl acetone, β-ionone	Foods
Syzygium aromaticum (=*Eugenia aromatica*)	(*E*)-β-Ocimene, linalool, terpinen-4-ol, nerol	Food, beverages, spice (meat, curry) Analgesic, anticancer, antimicrobial, antioxidant, antiseptic, hypoglycemic, lower blood sugar For stomach ulcers
Taraxacum officinale	Carvacrol, dihydrocitronellal (**54**), geranyl acetone	Foods, beverages Antiinflammatory, antioxidant, antiviral, blood and lipid regulatory, diuretic, hepatoprotective For breast and gallbladder problems, infections, pneumonia, swelling, stimulating bile flow, water retention
Taxus cuspidata	Linalool, α-terpineol, myrtenal, geraniol, nerol, bornyl acetate, *p*-cymen-9-ol	Beverages (herbal tea) Antihypertension For heart, kidney and liver disorders, paralysis, neuralgia rheumatism

(continued)

Table 1 (continued)

Thea sinensis (Green tea oil)	Linalool, geraniol	Beverages, food additives (ice cream, noodle) Antihypertension, antiobesity, antioxidant, antiviral, hypocholesterolemia For blood clots, blood sugar control, cavities, colds, flu, gum and heart disease, immunity system booster, osteoporosis, ulcers
Thujopsis dolabrata var. *hondai* (Hiba oil)	α-Pinene, sabinene, β-pinene, limonene, myrcene, borneol, sabinol, sabinyl acetate, terpinyl acetate carvacrol, α-thujaplicin (**327**), β-thujaplicin (**328a**), γ-thujaplicin (=hinokitiol) (**328b**)	Soap flavor, aroma retaining agents Antibacterial, antifungal, antiinflammatory, antiproliferative, antiviral For hypertension, liver and kidney disorders, jaundice rheumatoid
Thymus quinquecostatus	Tricyclene, thujene, α-pinene, camphene, myrcene, limonene, terpinolene, Δ^2-carene, *p*-cymene, (*E*)-β-ocimene, γ-terpinene, linalool, terpinen-4-ol, nerol, geraniol, (*Z*)-piperitol (**263**), borneol, bornyl acetate, camphor, dihydrocarvone, carvone, thymol, carvacrol, thymol methyl ether, linalyl butanoate (**170**)	Foods, beverages, cosmetics, pharmaceutics Antimicrobial, antitumor Insecticide, mosquito repellent
Thymus vulgaris (Thyme oil)	α-Thujene, α-pinene, β-pinene, camphene, verbenene (**341**), sabinene, myrcene, α-phellandrene, Δ^3-carene, limonene, α-terpinene, *p*-cymene, 1,8-cineol, (*E*)-β-ocimene, γ-terpinene, camphor, linalool, terpinen-4-ol, α-terpineol, (*E*)-sabinene hydrate (**296**), (*Z*)-linalool oxide, (*E*)-linalool oxide, (*E*)-verbenol, borneol, geraniol, *p*-cymen-9-ol, verbenone, thymol methyl ether, linalyl acetate, fenchone, thymol, carvacrol	Food flavor (source, dressing), dried leaf seasoning, crude drugs Anticancer, anticonvulsant, antifungal, antioxidant, antiseptic, antitussive, carminative, expectorant, rubefacient
Tragopogon porrifolius (Salsify)	Limonene, geranial, geranyl acetone	Foods, for soups and stews
Tricholoma equestre	α-Pinene, limonene, 1,8-cineol, linalool, geranyl acetone, β-ionone	Foods
Tricholoma matsutake (Matsutake fungus)	α-Pinene, limonene, linalool, α-terpineol, bornyl acetate	Foods, for soups Anticancer, antimicrobial

(continued)

Table 1 (continued)

Tricholomopsis rutilans	α-Pinene, β-pinene, 1,4-cineole, limonene, 1,8-cineol, linalool, geranyl acetone, β-ionone	Foods
Trigonella foenum-graecum (Fenugreek)	*Dried seeds*: α-thujene, α-pinene, sabinene, limonene, 1,8-cineole, linalool, borneol, terpinen-4-ol	Foods, vegetable, curry powder Analgesic, antiobesity, antipyretic, diuretic, gastroenteritis, hypocholesterolemia, oral diseases, stomachic
Trigonella torbatjamensis	α-Pinene, sabinene, limonene, 1,8-cineole, α-thujone, borneol, terpinen-4-ol, α-terpineol, carvone, bornyl acetate	Vegetable Antipyretic, diuretic For anorexia, bronchitis, chronic, colitis, cough, dropsy, hernia supportive, heart disease, impotence, vomiting
Triticum aestivum (Bread wheat)	Limonene, (*E*)-β-ocimene, (*Z*)-β-ocimene, linalool, linalool oxide	Foods, beverages Anticancer, antiinflammatory For childhood asthma, preventing gallstones, post-menopausal symptoms, reducing chronic inflammation
Ulva pertusa (Sea lettuce, green laver)	α-Pinene, limonene, *p*-cymene, *m*-cymene, linalool, perilla alcohol, perillaldehyde, carveol, β-cyclocitral, α-ionone, β-ionone, geranyl acetone	Foods, food additives, beverages
Undaria pinnatifida	β-Cyclocitral, α-ionone, β-ionone	Foods, food additives, beverages Antiangiogenic, anticancer, antiinflammatory, antiobesity, antioxidant, neuroprotective For cerebrovascular diseases
Urtica dioica	Camphor, borneol, menthol, β-cyclocitral, β-homocyclocitral (**74**), bornyl acetate, thymol, carvacrol	Foods, vegetable Antiasthmatic, antidandruff, antifungal, antioxidant, antiviral, astringent, depurative, diuretic, galactagogic, hemostatic, hypoglycemic, immunomodulatory, tonic For anemia, arthritis, eczema, excessive menstruation, hematuria, hemorrhoids, jaundice, menorrhagia, nephritis, rheumatism

(continued)

Table 1 (continued)

Valeriana hardwickii	α-Pinene, camphene, γ-terpinene, α-terpinyl acetate, bornyl acetate	Beverages Analeptic, antidiarrheal, antispasmodic, carminative, diuretic, expectorant, hypotension, nervine, nerve tonic, sedative, stimulant For epilepsy, hysteria, rheumatic
Valeriana officinalis (Valerian oil)	Tricyclene, α-thujene, α-pinene, α-fenchene, camphene, sabinene, β-pinene, *p*-cymene, limonene, β-phellandrene, γ-terpinene, camphor, terpinene-4-ol, α-terpineol, myrtenol, (*E*)-carvacrol, borneol, bornyl acetate, (*E*)-pinocarveyl acetate (**259**), myrtenyl acetate, terpinyl acetate, (*Z*)-carveyl acetate (**39b**), linalyl *iso*valerate (**172**), β-ionone, bornyl *iso*valerate (**17**), myrtenyl isovalerate (**198**), neryl *iso*valerate (**237b**), geranyl isovalerate (**104**), perillaldehyde, geranyl pentanoate (**103**)	Food flavor, beverages Anodyne, antiasthma, anticonvulsant, antihypertension, antispasmodic, antitussive, carminative, hypnotic, sedative, diuretic For cramp, excitability, hypochondriasis, hysterical states, intestinal colic, insomnia, migraine
Valeriana officinalis var. *latifolia*	Tricyclene, α-pinene, Δ^3-carene, α-fenchene, camphene, β-pinene, β-terpinene, myrcene, *p*-cymene, limonene, β-phellandrene, γ-terpinene, terpinolene, linalool, (*E*)-*p*-mentha-2,8-dien-1-ol, limonene, camphor, terpinen-4-ol, α-terpineol, *p*-menth-1-en-8-ol, borneol, myrtenol (=2-pinen-10-ol) (**196**), perillaldehyde, bornyl acetate, myrtenyl acetate, terpinyl acetate, *iso*-bornyl isovalerate (**21**), (*E*)-verbenyl acetate (**339b**)	Food flavor Antiasthma, anticonvulsant, antitussive, diuretic, sedative, uretic
Verbena officinalis	Limonene, 1,8-cineole linalool, verbenone, geranial	Beverages (herbal tea) For menstrual dysfunction
Vicia faba (Broad bean)	3-Thujen-2-ol (**332**), β-damascone	Foods Antioxidant, bone strengthening For anemia

(continued)

Table 1 (continued)

Vigna angularis (Azuki)	Linalool, linalool oxide, (*Z*)-jasmone (**133**), α-ionone, β-ionone, damascone	Foods Antiadipogenic, antioxidant
Vitis vinifera	Linalool, nerol, citronellol, α-terpineol, (*E*)-linalyl oxide, (*Z*)-linalyl oxide, geranial, geraniol, 8-hydroxydihydrolinalool (**161**), hydroxynerol (**239**), (7*E*)-8-hydroxylinalool (**159a**), hydroxygeraniol (**112**), geranic acid	Foods, fruit, jams, juice, beverages (wine) Antiinflammatory *Seed extract:* anticancer, increase HDL cholesterol For allergy symptom, arteriosclerosis, diabetes, endometriosis, fibromyalgia, heart disease, hemorrhoids, psoriasis, retinopathy
Xanthium strumarium	Limonene, borneol	Beverages (herbal tea) Analgesics, anodyne, anthelmintic, antipyretic, antirheumatic, sedative, tonic For appetizer, complexion cooling, diaphoretic, digestive, diuretic, emollient fattening, improving appetite, laxative, neuralgia, sweating
Xerocomus subtomentosus	Limonene	Foods
Zanthoxylum piperitum (Japanese pepper oil)	α-Pinene, β-pinene, sabinene, myrcene, limonene, α-phellandrene, (*Z*)-β-ocimene, (*E*)-β-ocimene, Δ^2-carene, β-phellandrene, 1,8-cineol, α-terpinene, terpinolene, *p*-cymene, linalool, citronellol, citronellal, *iso*-pulegol, β-terpineol, α-terpineol, nerol, geraniol, terpinen-4-ol, cryptone, citronellol, cumin aldehyde, geranyl acetate, neryl acetate, citronellyl acetate, piperitone, *iso*-pulegol, (*E*)-*p*-mentha-2,8(9)-diol (**219**), myrtenol, (*Z*)-carveol, (*E*)-carveol, 18-hydroxylinalool, linalyl acetate, linalool 8-acetate (**160**), α-terpinyl acetate	Foods, food additives, spice (young leaf, seed) Anthelmintic, antibacterial, antifungal, antiinflammatory, antimicrobial, antioxidant, antiperiodic, antiseptic, antispasmodic, antitumor, antitussive, astringent, carminative, digestive, disinfectant, diuretic, expectorant, immunity booster, parasiticide, stimulant, stomachic, tonic For poor circulation, sweating
Zea mays (Maize, corn)	Myrcene, limonene, thymol, carvacrol, geranyl acetone	Foods
Zingiber mioga (Mioga ginger)	α-Pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, limonene, β-phellandrene, 1,8-cineole, (*E*)-ocimene, (*Z*)-ocimene, *p*-cymene, γ-terpinene, (*E*)-	Foods, food additives Anticancer, antihypertensive, antiinflammatory, antiviral For abnormal and irregular digestion, fever, hematemesis, menstruation, skin health

(continued)

Table 1 (continued)

	sabinene hydrate, linalool, (*Z*)-sabinene hydrate, terpinen-1-ol (**306**), α-fenchyl alcohol, β-terpinene (**307c**), *neoiso*-menthol, pinocarveol, α-terpineol, borneol, myrtenol, (*E*)-carveol, *p*-cymen-9-ol, *p*-menth-3-en-7-ol (**223**), β-cyclocitral, camphor, linalyl acetate, limonene-1,2-epoxide, perillaldehyde, carvenone (**36**), piperitone, carvone, bornyl acetate, methyl geranate, myrtenyl acetate, β-cyclocitral, myrtenal, citral, fenchone, thymol, geranyl acetone, β-ionone	
Zingiber officinale	*Fresh rhizome*: β-pinene, camphene, myrcene, β-phellandrene, (*Z*)-β-ocimene, *p*-cymene, 1,8-cineol, (*Z*)-sabinol *p*-cymen-9-ol, camphor, α-phellandrene, terpinolene, α-terpineol, linalool, *iso*-borneol, neral, geranial, geranyl acetate, *iso*-bornyl formate (**19b**) *Steam distilled*: tricyclene, α-pinene, camphene, sabinene, myrcene, β-pinene, γ-terpinolene, linalool, camphor, camphene hydrate (**24**), terpinen-4-ol, citronellal, borneol, α-terpineol, citronellol, neral, geraniol, geranial, citronellyl acetate, geranyl acetate, geranic acid	Foods, food additive (bread, cookies, source, liqueurs, candied food), spice, beverages (ginger ale) Analgesic, anticancer, antifungal, antimicrobial, antiinflammatory, antioxidant, carminative, diaphoretic, diuretic, GI irritant, insecticidal, mild stimulant For back pain, cold, gastrointestinal and respiratory disease, detoxification, nausea, nephroprotective, relaxant, stomachic, sweating, warming
Zingiber zerumbet	α-Pinene, camphene, sabinene, myrcene, limonene, l,8-cineole, linalool, γ-terpinene, geranial	Foods, food additives, beverage Antiallergic, analgesic, antimicrobial, antinociceptive, immunomodulatory
Ziziphus jujuba	(*E*)-β-Ocimene, perillene (**277**), γ-terpinene, linalool, *(Z)*-sabinene hydrate, (*E*)-sabinene hydrate, terpinen-4-ol, citronellol	Foods, fruit, beverages, for Kampo medicine Anticancer, antiinflammatory, antiobesity, antioxidant, hepato- and gastrointestinal protective, immunostimulating, inhibition of foam cell, formation in macrophages

known, and these constitute acyclic, monocyclic, and bicyclic monoterpenoids. Monoterpenoids occur in nature as hydrocarbons, alcohols, aldehydes, ketones, oxides, carboxylic acids, and their esters including lactones.

As seen in Table 1, almost all of the listed foods and beverages contain monocyclic monoterpenoids with *para*-menthane skeleton. Acyclic and bicyclic monoterpenoids have been found in foods as secondary major components. Many *Citrus* species belonging to the Rutaceae, *Curcuma* and *Zingiber* to the Zingiberaceae, and *Cinnamomum* to the Lauraceae families contain a large number of monoterpenoids in their peels, rhizomes, leaves, and/or roots. Foods belonging to the Lamiaceae also include many monoterpenoids. 170 edible species of the Lamiaceae and their medicinal properties due to biological activity were reviewed including the references (Carovic-Stanko et al. 2016). Many of them have also been listed in Table 1. They are *Acinos* (2 species), *Ajuga* (2), *Anisochilus* (2), *Anisomeles* (1), *Agastache* (1), *Calamintha* (3), *Cedronella* (1), *Clerodendrum* (1), *Coleus* (4), *Dracocephalum* (1), *Elsholtzia* (4), *Glechoma* (1), *Gmelina* (1), *Gomphostemma* (2), *Hyptis* (5), *Isodon* (3), *Lallemantia* (1), *Lamium* (4), *Lavandula* (8), *Leucas* (4), *Leonotis* (1), *Lycopus* (2), *Meehania* (1), *Melittis* (1), *Mentha* (13), *Micromeria* (3), *Monarda* (4), *Mosla* (2), *Nepeta* (4), *Ocimum* (4), *Origanum* (5), *Perovskia* (1), *Phlomis* (4), *Plectranthus* (1), *Pogostemon* (1), *Salvia* (16), *Satureja* (5), *Scutellaria* (1), *Sideritis* (3), *Stachys* (23), *Teucrium* (2), *Thymra* (1), *Thymus* (14), and *Ziziphora* (1).

Over 200 herbs and species including foods and medicinal uses with beautiful illustration of each plant species have been demonstrated by Ravindran (2017).

The most common monoterpene hydrocarbons are α-pinene (**254**), β-pinene (**255**), camphene (**23**), myrcene (**191**), limonene (**152**), terpinolene (**316**), α- (**256**) and β-phellandrene (**257**), *p*-cymene (**79**), and (*E*)- (**248**) and (*Z*)-β-ocimene (**249**), of which limonene is a ubiquitous component in various *Citrus* fruits. α-Terpinene (**308**) and γ-terpinene (**309**) are often found in vegetable and fruits. α- (**329**) and β-thujenes (**330**), fenchene (**88**), sabinenes (**294** & **295**), and tricyclene (**336**) are rare monoterpene hydrocarbons in foods and fruits. The monoterpene ketones, camphor (**29**), carvone (**42**), piperitenone (**275**), and piperitone (**281**), are often seen in foods as minor components. The presence of monoterpene alcohol, menthol (**183**), is limited in the genus *Mentha* as the major compounds, together with its stereoisomers, *iso*-menthol (**186a**), *neo*-menthol (**187**), and *neoiso*-menthol (**188**) and menthone (**189**). Borneol (**13**), geraniol (**97**), linalool (**158**), nerol (**234b**), terpinen-4-ol (**307a**), and α-terpineol (**312**) are widely distributed monoterpene alcohols in many foods. In *Citrus* group, a few monoterpene aldehydes, citronellal (**55**), geranial (**94**), and neral (**294a**), have been found as the major components to contribute to their characteristic aroma. The *Perilla* genus produces perillaldehyde (**274**) and its dihydro derivative such as *(Z)*- (**303**) and *(E)*-shisools (**304**) (Almed 2019). The most predominant monoterpene ethers are 1,8-cieole (**52**) and linalool oxides (**164,a**, **164b**, **165a** & **165b**). As the monoterpene esters, bornyl (**15**), citronellyl (**59**), geranyl (**99**), and terpinyl acetates (**313**), have been found in

many different foods and vegetables, only several aromatic monoterpenoids, such as carvacrol (**34**) and thymol (**333**), and their methyl ethers (**35**, **334**), cumin alcohol (**70**), and cumin aldehyde (**72**) as well as cymene derivatives (**76-78** & **80-83**) have been isolated from or detected in the *Thymus* and the other Lamiaceae species as the main phenolic monoterpenoids. There are many characteristic fruits and vegetables including totally different monoterpenoids from the other edible plant groups. They are *Actinidia polygama* which included actinidin (**1**), actinidiolide (**2**), matatabiether derivatives (**176-179**), and *neo*-matatabi lactone (**238**). *Aralia elata*, *Daucus carota*, *Pelargonium graveolens*, and *Valerian officinalis* produce many alkynyl esters of borneol (**14-21**), citronellol (**59-66**), geraniol (**98-110**), linalool (**167-174**), lavandulol (**137-140**), and nerol (**235-238**), respectively. *Nepeta cataria* is also very specific medicinal plant biosynthesizing monoterpene with δ-lactone skeletons, such as nepetalactone derivatives (**226-229**, **231**). The chemical profiles of *Perilla* species used as the vegetable in the Far East are also very characteristic because they produce not only perillaldehyde (**274**) and its monodihydro derivatives, shisools (**303** & **304**), mentioned above but also different types of monoterpene ketones from the other ones, such as *iso*-egomaketone (**87a**), elsholtzia ketone (**87b**), naginata ketone (**224**), and perilla ketone (**273**). Theaspirane (**319**) and its derivatives (**319-320**), having unique spiro skeletal structures, were detected in *Pseudocydonia sinensis* and *Rubus idaeus*. *Chenopodium ambrosioides* elaborates a few unique endoperoxides, ascaridole (**9**) and its dihydro derivative (**10**), as well as two *p*-menthane diepoxides (**11a** & **11b**).

Zingiber officinale is one of the most widely used as foods, food additives, spice, and beverage (ginger ale and herbal tea) in the world. In Japan sliced fresh ginger with vinegar is important diet for Sushi, and its dried powder with sugar has been used as antitussive and warm protection of the body, and a great number of the references of phytochemicals and their benefits of *Z. officinale* are in many journals (Sharifi-R et al. 2017). Its essential oils contain not only pungent substances, gingerols and shogaol, but also a number of different monoterpenoids are identified.

One of the most widely used spices is the *Curcuma* species, of which *C. longa* (=*C. domestica*) is the most popular food in the world, and curcumin, yellow pigment in its rhizome, has been used for Cali in particular. There are so many *Curcuma* species in Asia, and they have traditionally been used as folk medicines and foods as well as beverages as shown in Table 1 (Dosoky and Setzer 2018, Dosoky et al. 2019; Zhang et al. 2017; Rajkumari and Sanatombi 2017; Widyowati and Agil 2018; Zachariah and Leela 2006; https://www.newdirectionsaromatics.com/blog/products/).

The *Cinnamomum* species are also widely used as herbal tea as well as Kampo medicine for cold, flu, etc. (Liyanage et al. 2017; Zachariah and Leela 2006). One of the most widely used fruits as foods, desserts, and beverages is the *Citrus* species which contained monoterpenoids as major volatiles (Gonzalez-Mas et al. 2019; Njorogo et al. 1994; Tomiyama et al. 2012). There are so many biodiversity of *Citrus*, of which *Citrus lemon* in Europe, *C. aurantium* (Mexican lime), *C. sinensis* (Valencia orange), and *C. paradisi* (grapefruit) are well-known species and so important for diet. *C. sudachi* which is endemic in Tokushima Pref. in Japan;

C. junos (Yuzu) in Kochi, Tokushima, and Kyoto; and *C. sphaerocarpa* (Kabosu) in Oita, Japan, are very well-known fruits, for application to many Japanese cuisines, especially to Sushi, Tempura, and noodles. Recently there are also many hybrids of *Citrus* fruits which are often much more tasty or aromatic than each mother *Citrus* species.

One of the most widely used dietary monoterpenoids is (−)-menthol (**183**) which has been obtained by steam distillation of several *Mentha* species as the natural products; however, environment-friendly green or clean chemistry is emphasized in the field of organic and natural product chemistry. Noyori's high efficient production of (−)-menthol using (S)-BINAP-Rh catalyst is one of the most important green chemistries (Tani et al. 1982; Otsuka and Tani 1991), and 1,000 ton of (−)-menthol has been produced by this method for 1 year and used as food additives, ice cream, candies, chewing gums, cosmetics, toothpaste, shampoo, etc.

The benefits including the application of foods and food additives as well as the medicinal uses of the representative volatile oils from basil, bergamot, camphor, carrot, cedar wood, citronella, clary, eucalyptus, frankincense, geranium, ginger root, lavender, lemon, lemongrass, myrrh, orange, patchouli, pine, rose geranium, rosemary, sage, spearmint, tea tree, turmeric, vetiver, wintergreen, and ylang-ylang are summarized in the website (https://www.newdirectionsaromatics.com/blog/products/). The top 12 healthful fruits are also shown in the website (http://medicalnewstoday.com), and 11 best low-sugar fruits, 10 low-glycemic fruits for diabetes, 11 best fruits for weight loss, 20 top healthy salad topping, 20 healthiest fruits on the planet, and 10 low-carb smoothies preparation related to the dietary monoterpenoids are reported in the website (http://healthline.com). The Useful Tropical Plants Database (http://tropical.theferns.info/viewtropical.php) contains the information on the edible, medicinal, and many other uses of several thousand plants that can be grown in tropical regions.

16.3 Distribution of Monoterpenoids and Related Compounds in Spore-Forming Plants and Fungi

Sea Algae: Seaweeds have been used for centuries for both food and medicinal purposes (Santos et al. 2018). It has been long believed that they possess antiaging properties and help to keep good health. As a great number of chemical researches have been carried out on various types of seaweeds over the years, the abundance of antioxidants has been found in many sea algae. There have also been some important discoveries of antiinflammatory and immune system booster properties from sea algae. Scientists have studied the three main varieties of sea algae, green (Chlorophyceae), brown (Phaeophyceae), and red species (Rhodophyceae). Several sea algae, green alga, *Ulva pertusa*; brown algae, *Undaria pinnatifida* and *Saccharina japonica* (= *Laminaria japonica*); and red alga, *Pyropia tenera*, have been used as foods and processed foods in the Far East, especially in Japan, almost every day; however, the presence of monoterpenoids in algae is very rare. *U. pertusa* contains several monoterpene hydrocarbons, α-pinene, limonene, and *p*- and *m*-

cymenes, as well as perilla alcohol (**271**) and its aldehyde (**274**) which have been found in the terrestrial vegetables, such as *Perilla* species. *Saccharina* and *Pyropia* species produce *a*-terpineol (**312**).

Bryophytes: More than 20000 species of bryophytes (mosses, liverworts, and hornworts) are known. However, neither references of dietary monoterpenoids nor those who ate such a small plant group have been seen since a long time ago. Only liverworts contain oil bodies in organelle cell, and a large amount of mono-, sesqui-, and diterpenoids, as well as aromatic compounds and polyketides, have been found (Asakawa 1982, 1995; Asakawa et al. 2013). Recently one of the thalloid liverworts, *Conocephalum conicum*, which contains several monoterpene hydrocarbons and alcohol has been served as toping to hamburger and to consommé soup in Japan.

***Pteridophytes*:** There are a great biodiversity of ferns. Generally the leaves and stems of ferns are very solid, and they are not edible except for several species. In Japan three species of young shoots of a few ferns, *Equisetum arvense*, *Pteridium aquilinum*, and *Osmunda japonica*, are served as foods and vegetables; however, the presence of ubiquitous monoterpene hydrocarbons and alcohols is seen in the former two species as shown in Table 1.

***Lichens*:** This group is also large biodiversity. Since very early times, many lichens have been used to cure diarrhea, epilepsy, fever, hydrophobia, jaundice, and skin disorders. Some lichens have been known to have anticancer and spasmolytic activity. One of the most economical lichens is *Evernia prunastri* from which essential oils are obtained as the preparation of cosmetics and perfumery goods. This lichen also possesses antiinflammatory, antiirritant, antiseptic, and expectorant properties and is treated for intestine and skin disorders. It contains ubiquitous monoterpenoids as seen in Table 1. However, no records as a food of this species have been seen in the references. On the other hand, *Cetraria islandica* and *Bryoria fremontii* were important sources of food for humans in Northern Europe and North America in the past, respectively. *Umbilicaria* and *Lasallia* have frequently been used as foods in North America; *Umbilicaria esculenta* is used in a variety of traditional Korean and Japanese foods. However, the above lichens do not contain any monoterpenoids.

***Fungi and Mushrooms*:** The phytochemicals of fungi and mushrooms are also a great biodiversity and very complex (Pavel 2016). There are three kinds of mushrooms, edible, inedible, and poisonous species, of which 8 million tons of edible and nutritional mushrooms are consumed as foods for 1 year in the world. Several edible mushrooms *Boletopsis leucomelas*, *Clitocye odora*, *Flammulina velutipes*, *Grifola frondosa*, *Lentinus edodes*, *Pleurotus ostreatus*, *Pholiota microspora*, and the other species contain monoterpenoids with n-octane derivatives (Kalac 2016; Parior et al. 1996a, b). However, only several edible mushrooms containing monoterpenoids are served for European, Chinese, and Japanese cuisine. For example, black fungus, *L. edodes*, has been used as many foods and processed foods; however, the content of the monoterpenoids, both the fresh and dried fruit bodies, is faint. The most expensive mushroom in Japan is *Tricholoma matsutake* which is used for consommé soup. It produces 1-octen-3-ol and methyl cinnamate which are important aroma of this mushroom with a few monoterpenes, limonene, linalool, and α-terpineol, as the

minor components. Essential oil derived from the fungus, *Ceratocystis virescens*, contains α-terpineol, linalool, geranial, geraniol, geranyl acetate, neral, citronellol, and citronellyl acetate. *Trametes odorata* has been reported to have citronellol, nerol, and geraniol. Some *Phellinus* species produced linalool. The volatile oil of the rust fungus, *Cronartium fusiforme*, includes α-pinene, β-pinene, camphene, 3-carene, limonene, β-phellandrene, and terpinen-4-ol; however, these fungi have not been used as foods. Several edible and nutritional mushrooms including monoterpenoids are listed in Table 1.

16.4 Biological Activity of Monoterpenoids

Aerial parts, leaves, fruits, and flowers of the listed plants and spore-forming plants and fungi are edible. Tubers and roots of many plants are also edible. Almost all of the listed plants and fungi in Table 1 show various bio- and pharmacological activities, antimicrobial, anodyne, antiseptic, antiinflammatory, antiproliferative, anesthetic, antimalarial, antifungal, antirheumatic, excitant, laxative, anticholinesterase, diaphoretic, antiseptic, antiepileptic, febrifuge, anticancer, antitussive, hyaluronidase inhibitory, antimycobacterial, antioxidant, antipyretic, diaphoretic, gastroprotective, stomachic, anxiolytic, refrigerant, emmenagogue, cytotoxic, antispasmodic, analgesic, antidysentery, antihypertensive, antidepressant, ulcer healing, sedative, carminative, hypoglycemic, diuretic, astringent, cardiotonic, and tonic, and are used to treat colic, menstrual disorder, indigestion, flatulence, pulmonary and urinary infections, fever, cough, colds, cardiovascular and immunological diseases, chronic bronchitis, asthma, headache, fever, epilepsy, and dyspepsia. The most frequently cited activities in the listed medicinal plants are antimicrobial and antioxidant. Alcohol derivatives of oxygenated monoterpenoids had greater antimicrobial activities than those of ketone derivatives. For example, menthol inhibited the growth of 24 bacterial strains, whereas menthone showed antifungal activity against only 4 bacteria. The similar phenomenon was also found for fenchyl alcohol and fenchone. Borneol, linalool, and nerol were more active than their acetate derivatives (Kotan et al. 2007).

Cancer is the second largest single cause of death climbing over six million lives every year worldwide. Colorectal cancer is one of the most common cancers in the world, and about 90% of this cancer is caused by metastasis, not by solid tumor. In spite of the recent chemotherapy development, survival times of cancer patients are very limited. Natural products including essential oils are the most successful strategy to find novel anticancer drugs and more than two thirds of the drugs used in cancer therapy.

Many diets including monoterpenoids show chemoprevention against cancer. Especially essential oils which are a mixture of volatile lipophilic constituents, monoterpenoids, and phenyl propanoids have potential anticancer and antitumor activities. More than several hundred papers have been published on anticancer and antitumor activity of essential oils and crude extracts from foods and fruits from

Table 2 Benefits and pharmacological activity of monoterpenoids included in foods, vegetables, and beverages

Monoterpenoids	Benefits and pharmacological activity
Ascaridole (**9**)	Anticancer (breast), anti-leishmanial, antimalarial, antinociceptive, antioxidant, antiparasitic, antitrypanosomal, antiviral, strengthen immune system
Borneol (**13**)	Anticancer, anesthetic, antispasmodic, antimicrobial, antiosteoporosis, sedative, skin tonic
Bornyl acetate (**15**)	Anticancer (gastric), analgesic, antifungal, antimicrobial, antiosteoporosis
Camphor (**29**)	Anticancer (colon), absorbent, anesthetic, antiarthritic, antifungal, antiinflammatory, antimicrobial, antineuralgic, antioxidant, antiosteoporosis, antiphlogistic, antirheumatic, antiseptic, antispasmodic, aphrodisiac, carminative, cooling, decongestant, diaphoretic, disinfectant, germicide, hypotension, narcotic, insecticide, nervous pacifier, treat cold, cough. For neurodermatitis, stimulant, warming
Carvacrol (**34**)	Anticancer (colon, lung), antibacterial, antifungal, antiinflammatory, antioxidant, antiparasitic, antiviral, strengthen immune system
Carveol (**39a** & **39b**)	Anticancer (breast), flavor additive
Carvone (**42**)	Anticancer (forestomach, lung), anesthetic, antiacetylcholinesterase, antiallergic, antifungal, antihyperglycemic, antioxidant, antiseptic, insecticidal, piscicidal, relaxant, expectorant, carminative, diuretic, sedative, stimulant, vermicide, perfume, flavoring
1,4-Cineole (**51**)	Antifungal
1,8-Cineole (**52**)	Anticancer (colon), analgesic, anthelmintic, antiacetylcholinesterase, antibacterial, anticaries, anticatarrhal, antifatigue, antifungal, antiinflammatory, antioxidant, antiosteoporosis, antiplaque modifiers halitosis, antirheumatic, antiseptic, antispasmodic, antitussive, antiulcer, antiviral, choleretic, convulsant, cough suppressant, decongestant, expectorant, headaches, hepatotonic, herbicidal, gastro protective, hypotension, increase cerebral blood flow, insecticidal, muscle relaxant, neurotoxin, pesticidal, sedative, skin absorption, stimulant, transdermal
Citral (**94** & **234b**)	Anticancer (breast), antibacterial, antifungal, antiviral, antiseptic, antioxidant. For physical or psychological stress, sedative
Citronellal (**55**)	Analgesic, antibacterial, antidepressant, antifungal, antiinflammatory, antimicrobial, antiseptic, antispasmodic, antiviral, diuretic, expectorant, febrifuge, hypertensive, pesticidal, insecticidal, sedative, stimulant, stomachic, tonic, vermifuge, flavor, fragrance
Citronellol (**58**)	Anticancer, analgesic, antifungal, antimicrobial, antiinflammatory, antiseptic, antispasmodic, anticonvulsant, hypotension, insecticidal, pesticidal, sedative, vasorelaxant

(continued)

Table 2 (continued)

Monoterpenoids	Benefits and pharmacological activity
p-Cymene (**79**)	Analgesic, antiacetylcholinesterase, antianxiety, antifungal, antimicrobial, antioxidant, neuroprotective, antiinflammatory, antirheumatic, antiviral, antiinfluenza, fragrance, herbicidal, laxative, pesticides, sedative, soothing
Dihydrocarvone (**43**)	Antimicrobial
Fenchone (**92b**)	Anticancer (breast, liver), antimicrobial, antifungal. For digestive discomfort
Fenchyl alcohol (**89** & **91**)	Antimicrobial, fragrance, flavoring agents
Geranial (**94**)	Rose-like scent: insecticidal, antioxidant
Geraniol (**97**)	Anticancer (colon, hepatoma, kidney, liver, lung, melanoma, pancreas, prostate, skin), analgesic, antibacterial, antioxidant, antiseptic
Geranyl acetate (**99**)	Antifungal, antimicrobial, antiinflammatory
Iso-menthol (**186a**)	Antimicrobial
Limonene (**152**)	Anticancer (breast, colon, forestomach, liver, lung, ovarian, prostate, skin), antiasthmatic, antifungal, antibacterial antihyperglycemic, antiinflammatory, antimutagenic, antiseptic, antispasmodic, antioxidant, antiviral, digestive, appetite suppressant, detoxicant, expectorant, herbicidal, immunomodulatory, muscle relaxant, pesticidal, transdermal absorption For physical or psychological, sedative, stress
Limonene 1,2-oxide (**154a** & **154b**)	Antimicrobial, antifungal, flavor
Linalool (**158**)	Anticancer, anesthetic, antiallergic, antiasthmatic, antispasmodic, antianaphylactic, anticonvulsant, antidepressant, antifungal, antiacetylcholinesterase, antileukemic, antimicrobial, antiinflammatory, antifungal, antimicrobial, antiseptic, antispasmodic, broncho relaxant, expectorant, narcotic, insecticidal, sedative
Linalyl acetate (**168**)	Anticancer (colon), antiinflammatory, antimicrobial, antiseptic, astringent, hypotensive, insecticidal
p-Mentha-2,8-dien-1-ol (**209a** & **209b**)	Anticancer (colon)
p-Mentha-8(9)-en-1,2-diol (=lemon diol) (**155**)	Anticancer (colon)
Menthol (**183**)	Anticancer (breast, colon), antiacetylcholinesterase, anesthetic, analgesic, antiallergic antiasthmatic, antibacterial, antihalitosis, antihistamic antimicrobial, antiinflammatory, antineuralgic, antirheumatic, antiosteoporosis, antipruritic, antipyretic, antiseptic, antispasmodic, bradycardic, carminative, cooling, decongestant, depressant, diaphoretic, expectorant, gastric sedative, modify dental pain, muscle relaxant, perfumery and flavoring, pesticidal, stimulant, refrigerant, respiratory
Menthone (**189**)	Antimicrobial
Myrcene (**191**)	Antiinflammatory, analgesic, antibiotic, antimutagenic, sedative

(continued)

Table 2 (continued)

Monoterpenoids	Benefits and pharmacological activity
Myrtenyl acetate (**197**)	Insecticidal
Nerol (**234b**)	Analgesic, antimicrobial, hypotension, tonic
Neryl acetate (**236**)	Antimicrobial, antiinflammatory, heal wound, healthy aging
Perilla alcohol (**271**)	Antiangiogenic, anticancer (breast, colon, liver, malignant brain, murine leukemia, ovarian, pancreas), antioxidant
Perillaldehyde (**274**)	Anticancer (colon)
Perillyl acetate (**272**)	Insecticidal, flavor, fragrance, insecticide
α-Phellandrene (**256**)	Antibacterial, antifungal, antimicrobial, diarrheal, emetic, hyperthermic, ingestion, laxative, pesticides, flavoring, perfumery
β-Phellandrene (**257**)	antifungal, antiviral, bactericidal, decongestant Expectorant, fragrance, perfumery, antiseptic
α-Pinene (**254**)	Anticancer, antibacterial, antiinfluenza, antiviral, antiinflammatory, antiosteoporosis, antiseptic, bronchodilator, expectorant, insecticide sedative, tranquillizer
β-Pinene (**255**)	Anticandidal
Piperitone (**281**)	Antiasthmatic, herbicidal, decongestant, flavoring, pesticidal. For synthesis of menthol
Sabinene (**294** & **295**)	Antifungal, antimicrobial, antiinflammatory, antioxidant
Terpinen-4-ol (**307a**)	Anticancer (colon), antiallergenic, antiasthmatic, antifungal, antimicrobial, antioxidant, antiseptic, antispasmodic, antitussive, antiacetylcholinesterase, antiulcer, diuretic, herbicidal, insecticidal, pesticidal, vulnerary
γ-Terpinene (**309**)	Anticancer (colon), antibacterial. Antifungal, antiinsomnia, antiproliferative, antioxidant, cytotoxic. For physical or psychological stress
α-Terpineol (**312**)	Anticancer, analgesic, antiesthetic, antiallergenic, antiasthmatic, antispasmodic, antitussive, antibiotic, antibronchitis, anticancer (colon), anticarcinogenic, anticonvulsant, antifungal, antihypertensive, antiinflammatory, antimicrobial, antinociceptive, antioxidant, antiulcer, cardiovascular disease, cholagogues, expectorant, insecticidal, perfumery, pesticides, skin penetration enhancing, sedative, vulnerary
Thujone (**331a** & **331b**)	Antiosteoporosis, insecticidal, convulsant
Thymol (**333**)	Anticancer (colon), antibacterial, antiobesity, antiosteoporosis, insecticidal, hypotension, immunity booster, pesticides, skin disorders
Thymoquinol	Anticancer (colon), insecticidal
Thymoquinone (**335**)	Anticancer (colon, myeloblast, leukemia HL-60, neuroblastoma), antiinflammatory, antioxidant, hepatoprotective, insecticidal
α-Thujaplicin (**327**) and β-Thujaplicin (**328a**)	Anticancer (colon), antiaging, antibacterial, antiinflammatory, antioxidant
Verbenone (**344**)	Anticancer, antimicrobial, antifungal, antioxidant, insecticidal, perfumery

which each monoterpene hydrocarbon and oxygenated monoterpene possessing antitumor and anticancer activity are listed in Table 2.

Bayala et al. (2014), Crowell (1999), and Sobral et al. (2014) reported the significant review articles of anticancer and antitumor active monoterpenoids from essential oils and solvent extracts. 1.8-Cineole (**52**), geraniol (**97**), limonene (**152**), perilla alcohol (**271**), perillaldehyde (**274**), and α-terpineol (**312**) showed effective for colorectal cancer in vitro and animal experiments. At present, the effect of chemoprevention against cancer and antitumor of essential oils have been investigated on breast, colon, brain, liver, skin, prostate, lung, leukemia, kidney, oral cervix, ovary, pancreas, mouth epidermal carcinoma, nasopharyngeal, neuroblastoma, stomach, and uterus, among which the breasts are the research target against anticancer of essential oils. One of the most treated monoterpenoids against cancer chemotherapy is geraniol (**97**) (Cho et al. 2016).

Many reviews concerning the cancer chemotherapy using essential oils and dietary monoterpenoids have been reported. Crowell (1999) summarized dietary monoterpene biochemistry, monoterpene metabolism and disposition, antitumor activity of monoterpenes, and mechanism of action of monoterpenes, referred by many recent papers. Biological property of α-terpineol was reviewed by Khaleel et al. (2018) in detail.

16.5 Microbial and Mammalian Biotransformation of Monoterpenoids and Their Related Compounds

As described in the former paragraph, not only fruits, vegetables, food, and products but also many herbal beverages contain many varieties of monoterpenoids, and we take these foods into our body, almost every day; however, the fetal of those volatile components in our body remained to be clarified. The entire major biochemical event taking place in an organism is controlled by enzymes which display major types of chemoselectivity, regio- and diastereoselectivity, and enantioselectivity. Enzymes of microorganisms and mammals are able to transform a huge variety of secondary metabolites, such as mono-, sesqui-, and diterpenoids, alkaloids, steroids, antibiotics, and amino acids, from foods, vegetables, and fruits as well as spore-forming green plants with the three major nutrients, carbohydrates, fats, and proteins, to produce pharmacologically and medicinally valuable substances. There are a great number of reports concerning biotransformation of essential oils, terpenoids, steroids, alkaloids, and acetogenins using bacteria, fungi, and mammals. Mikami (1988) reported the review article of biotransformation of terpenoids entitled "Microbial Conversion of Terpenoids." Noma and Asakawa (2010a, b) reported the biotransformation of a number of natural and synthetic acyclic and cyclic mono-, sesqui-, and diterpenoids, ionones, α-damascone, adamantanes, and aromatic compounds,

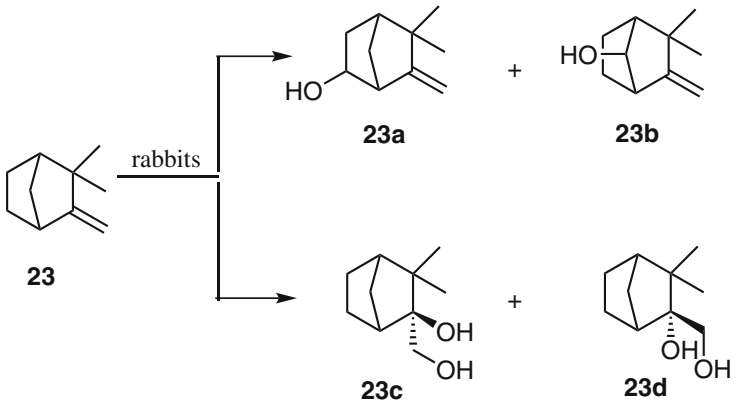


Fig. 1 Biotransformation of racemic camphene (**23**) by rabbits (Noma and Asakawa 2010a, b)

using mammals (rats and rabbits) and human enzymes. Biotransformation pathways of some representative monoterpenoids included in diets are shown in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 (Noma and Asakawa 2010a, b).

Camphene (**23**), Δ^3-carene (**31**), limonene (**152**), and (+)-α-pinene (**254**) and its enantiomer (**254**)′ were orally administered to rabbits, and after 1 day, urine was collected and then enzymatically hydrolyzed, followed by extraction with ether to obtain the biotransformed products. Rabbits converted camphene to two secondary alcohols (**23a** and **23b**) and two diols (**23c** and **23d**) (Fig. 1). Limonene (**152**) was biotransformed by rat CYP enzymes to give two natural products, (*E*)-carveol (**38b**) and limonene-10 (**156**) (Fig. 2).

Eight biotransformed compounds (**31a–31h**) were obtained from rabbits' urine after Δ3-carene (**31**) was orally administered. In this bioconversion, a three-membered ring was cleaved to give 2,2-dimethyl carbinol (**31a** & **31b**). Furthermore, one of 1,1-dimethyl group on a cyclopropane ring was oxidized to form primary alcohols (**31c**) and **31h.** The allylic methyl group at C-10 of **31** was also oxidized to give primary alcohol (**31f**), then to carboxylic acid (**31g**), followed by the further oxidation to afford **31h** (Fig. 3).

Both (+)- (**254**) and (−)-pinene (**254**)′ were independently administered to rabbits. From the latter substrate, an insect pheromone, (*E*)-verbenol (**342**′), was obtained in high yield. The human microsomal enzyme also converted to (−)-pinene to (*E*)-verbenol (**342a**′). Rabbits and human enzyme also converted both (+)- and (−)-pinene (**254** and **245**′) to myrtenol (**196**) and its enantiomer (**196**′) (Fig. 4). Terpinolene (**316**) was converted to two allylic primary alcohols (**316a**) and **316b** (Fig. 5).

Four monoterpene ketones, camphor (**29**), fenchone (**92**), menthone (**189**), and (−)-verbenone (**344**′), were treated with rat and human CYP enzymes, respectively. From (−)-camphor, (*Z*)-4-hydroxycamphor (**29a**) was obtained. On the other hand, three new hydroxycamphors (**29b**′**-29d**′) and an enantiomer (**29a**′) of **29a** were obtained (Fig. 6).

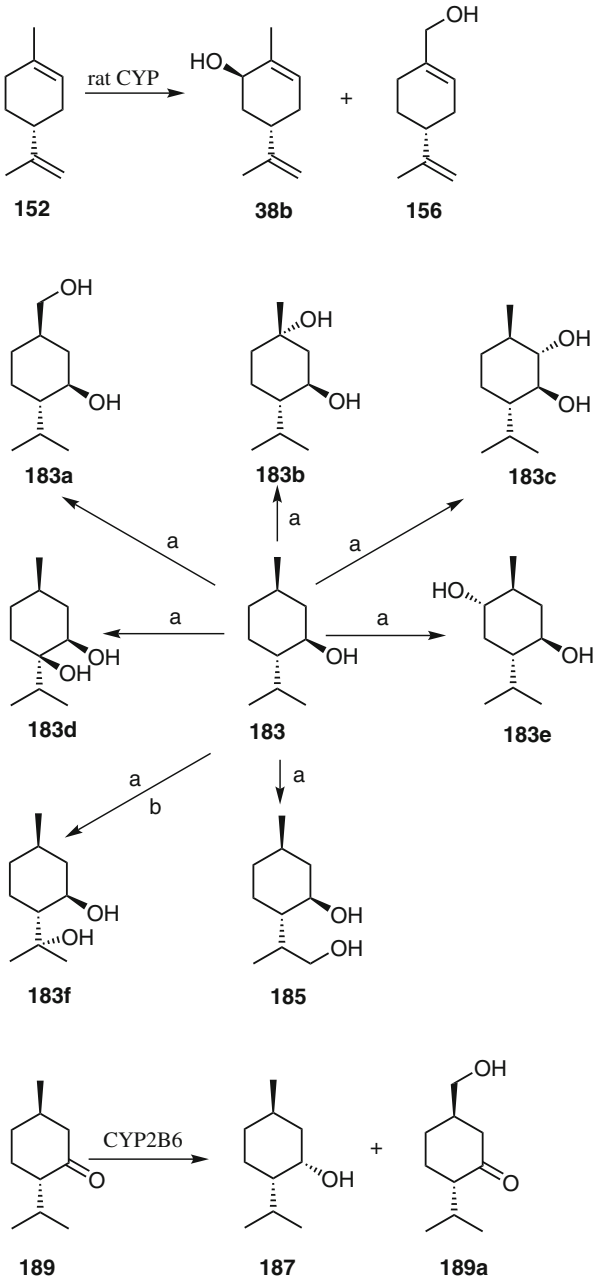


Fig. 2 Microbial biotransformation of (+)-limonene (**152**) by rat CYP2c11/CYP2B21, (−)-menthol (**183**) by *Aspergillus niger* (**a**) and human CYP 2A6 (**b**), and menthone (**189**) by CYP2B6 (Noma and Asakawa 2010a, b)

(+)-Fenchone (**92**) and its enantiomer (**92′**) were also treated with human CYP 450 to give two secondary alcohols (**92ba**, **92bb**) and one primary alcohol (**92bc**) and their enantiomers (**92ba′-92bc′**). In this case, enantioselectivity has not been observed (Fig. 7).

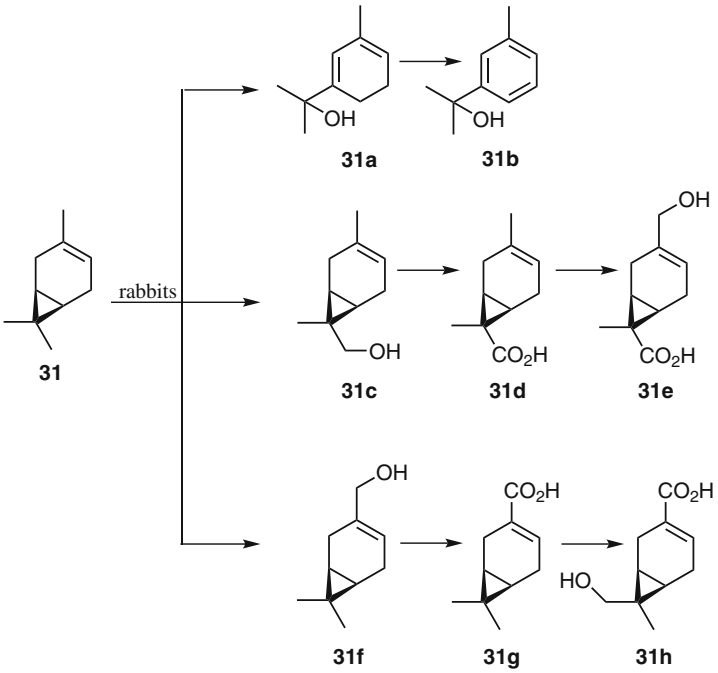


Fig. 3 Biotransformation of (+)-Δ^3-carene (**31**) by rabbits (Noma and Asakawa 2010a, b)

Treatment of menthone (**189**) with CYP2B6 enzyme gave 10-hydroxymenthone (**189a**) and (+)-*neo*-menthol (**187**). In this case, oxidation and reduction reactions occurred (Fig. 2). Treatment of (−)-verbenone (**344**) with human or rat CYP enzymes gave 10-hydroxyverbenone (**344a′**) and dihydroverbenone (**344b′**), respectively (Fig. 4).

(−)-Menthol (**183**) which is one of the most important dietary monoterpene alcohols was treated with human CYP enzyme or the black fungus, *Aspergillus niger*. The former enzyme biotransformed **183** only to 8-hydoxymenthol (**183f**) which possesses potent mosquito repellent activity. On the other hand, treatment of **183** with *A. niger* gave 6 hydroxymenthols (**183a-e**) and the same tertiary alcohol (**183f**) (Fig. 2).

Two monoterpene aldehydes, (−)-myrtenal (**195′**) and (*S*)-(−)-perillaldehyde (**274**), were orally administered to rabbits. Rabbit converted **195′** to myrtenic acid (**196c′**) and (−)-myrtenol (**196′**) which was further reduced to give two myrtanol isomers (**194a′** and **194b′**) (Fig. 4). From the latter compound, (*E*)-shisool (**303**) was obtained together with the carboxylic acid (**274a**) (Fig. 5).

Two monoterpene ethers, 1,4-cineol (**51a**) and 1,8-cineol (**52**), were also administered to rabbits. From the former urine, two tertiary alcohols (**51b** and **51c**), three primary alcohols (**51d–51g**), and a carboxylic acid (**51e**) which might be formed from the primary alcohol (**51d**) were isolated. 1.4-Cineol (**51**) was biotransformed by human and rat CYP enzyme to convert to (**2Z**)-hydroxy-1,4-cineole (**51a**)

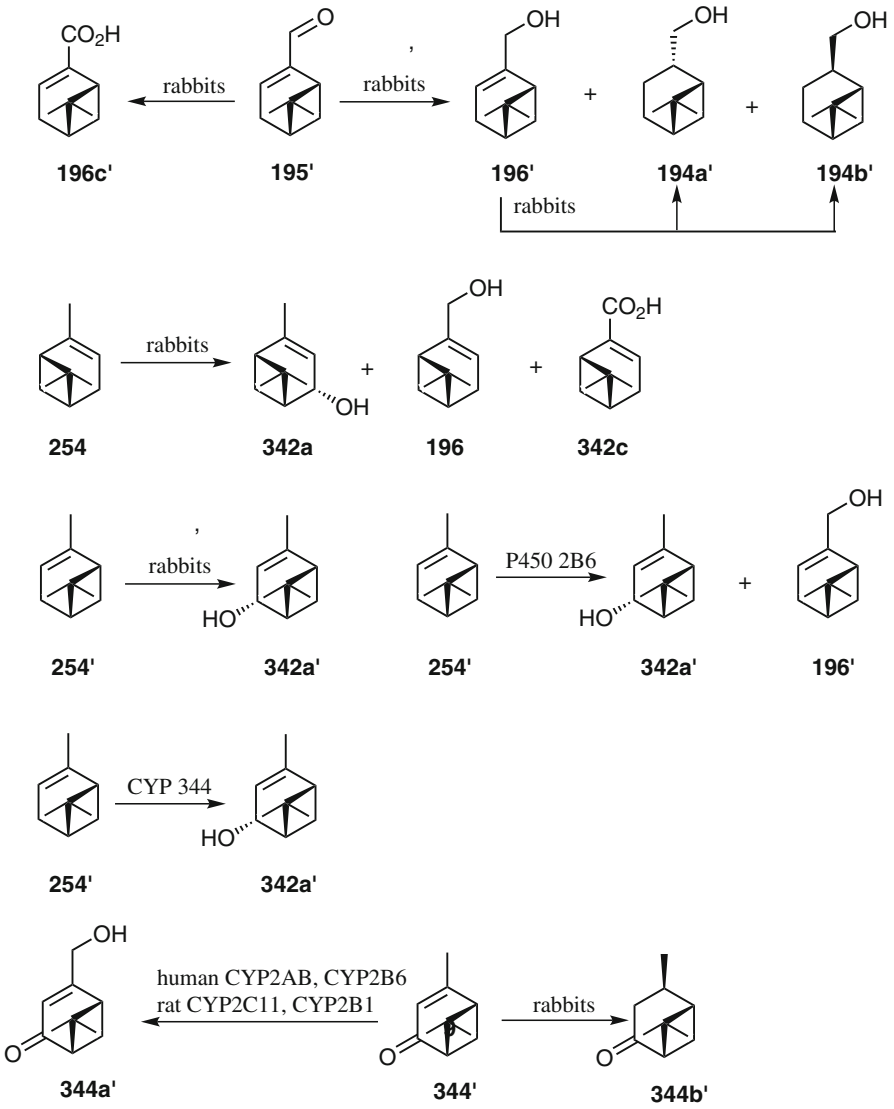


Fig. 4 Biotransformation of (−)-myrtenal (**195′**), (−)-mytenol (**196′**), (+)-α-pinene (**254**) and (−)-α-pinene (**254′**), and (−)-verbenone (**344′**) by rabbits and human liver microsomones (Noma and Asakawa 2010a, b)

(Fig. 8). On the other hand, rabbit converted 1,8-cineole (**52**) to four diastereoisomeric secondary alcohol (**51-52d**) but not to primary alcohol. In case of bioconversion of **52** using human enzyme and rats, three optical isomers (**52a′**, **52b′**, & **52d′**) of **52a**, **52b**, and **52d** were obtained in pure forms (Fig. 9). In this biotransformation,

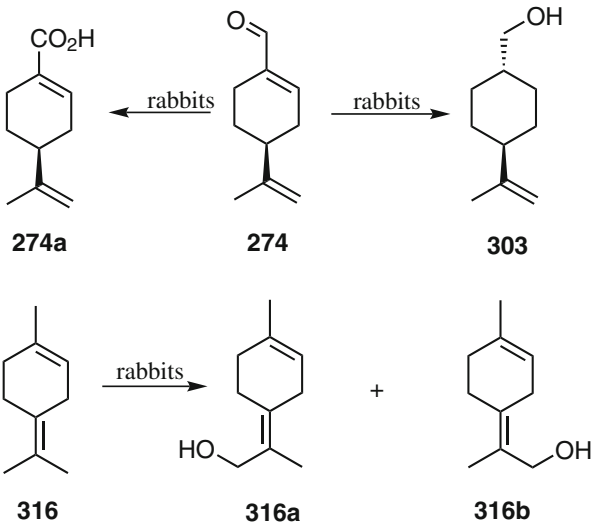


Fig. 5 Biotransformation of (*S*)-(−)-perillaldehyde (**274**) and terpinolene (**316**) by rabbits (Noma and Asakawa 2010a, b)

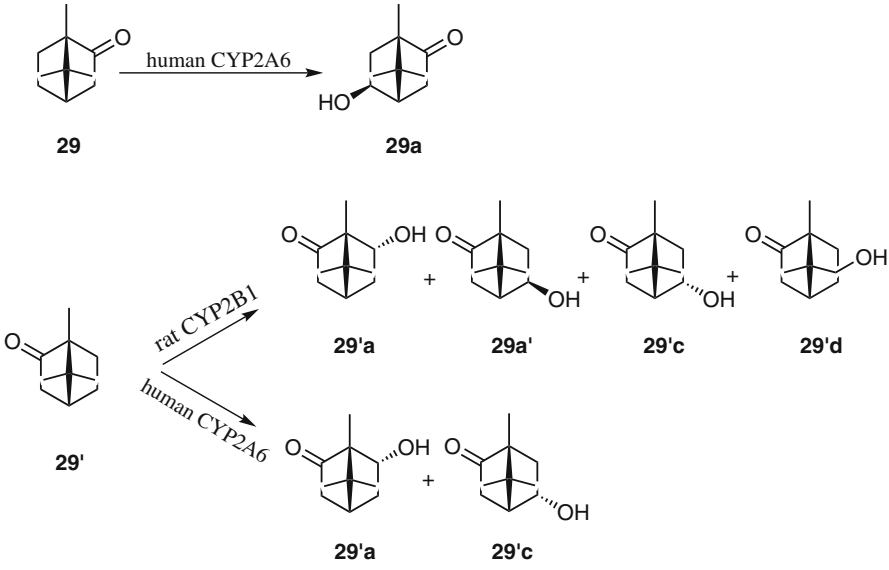


Fig. 6 Biotransformation of (−)-camphor (**29**) and (+)-(**29′**) by rat CYP 2B1 and human CYP2A6 rabbits (Noma and Asakawa 2010a, b)

the regiospecific introduction of oxygen function was observed by two enzymatic systems (rabbit and human/rat enzymes).

Two aromatic monoterpenoids, *p*-cymene (**79**) and carvacrol (**34**), were administered to rabbits or rats. From *p*-cymene, two natural products, 8-hydroxy-*p*-cymene

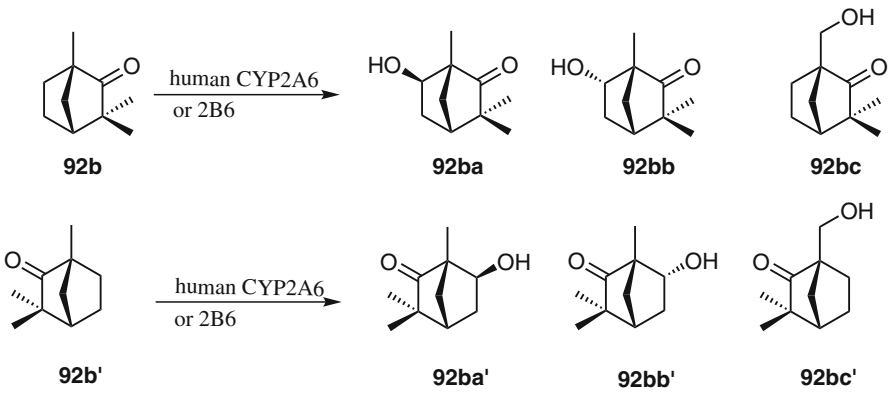


Fig. 7 Biotransformation of (+)-(**92b**) and (−)-fenchone (**92b′**) by human CYP2A6 or CYP2B6 (Noma and Asakawa 2010a, b)

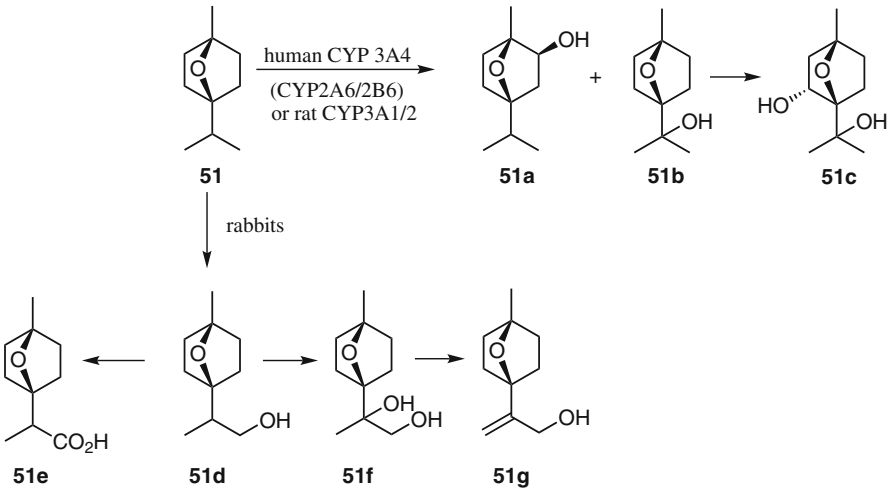


Fig. 8 Biotransformation of 1,4-cineole (**51**) by rabbits, human and rat P-450 enzymes (Noma and Asakawa 2010a, b)

(**80**) and 9-hydroxy-*p*-cymene (**81**), were obtained (Fig. 10). On the other hand, carvacrol (=2-hydroxy-p-cymene) (**34**) was administered to rat to give 8 metabolites (**14a-34h**), of which **34b** and **24e** possess the similar structures of **80** and **81** obtained from p-cymene (Fig. 10). The direct introductions of oxygen function on benzene ring and oxidation reaction of aryl methyl group have been observed in case of bioconversion of carvacrol (**34**). Thus almost all of monoterpenoids mentioned above were biotransformed by human enzymes and their direct administration by

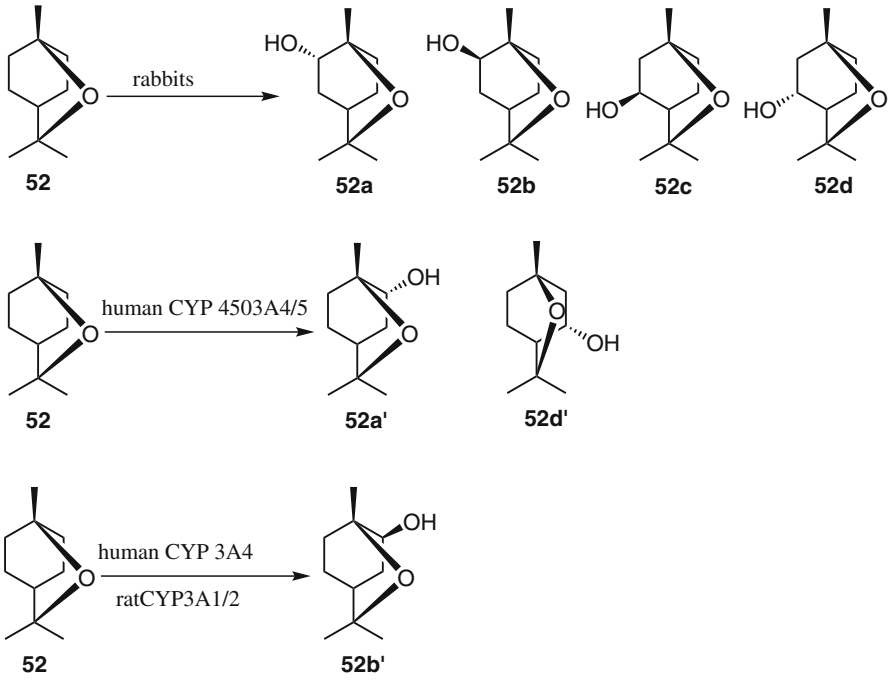


Fig. 9 Biotransformation of 1,8-cineole (**52**) by rabbits, human CYP 4503A/4/5, human CYP3A4 and rat CYP 3A1/2 (Noma and Asakawa 2010a, b)

mammals (rats and rabbits) to give oxidative metabolites, mainly primary, secondary, and tertiary alcohols. Such enzymes and mammals detoxify secondary metabolites, such as monoterpenoids, including foods, vegetables, fruits, and beverages, as well as processed foods and supplements.

16.6 Conclusion

As seen in Table 1, almost all of foods, vegetables, food additives, and beverage contain various types of monoterpene hydrocarbons, alcohols, ketones, and oxides. The presence of monoterpene aldehydes is restricted to be perillaldehyde, geranial, neral, and citronellal and cumin aldehyde. The presence of aromatic terpenoids is also limited to be *p*-cymene and its derivatives as well as carvacrol and thymol and their methyl ethers. The volatile components and essential oils are composed of a mixture of mainly monoterpenoids, and almost all of essential oils containing monoterpenoids show antimicrobial and antifungal activity. Such essential oils play an important role in the preserve of each fresh or dried food,

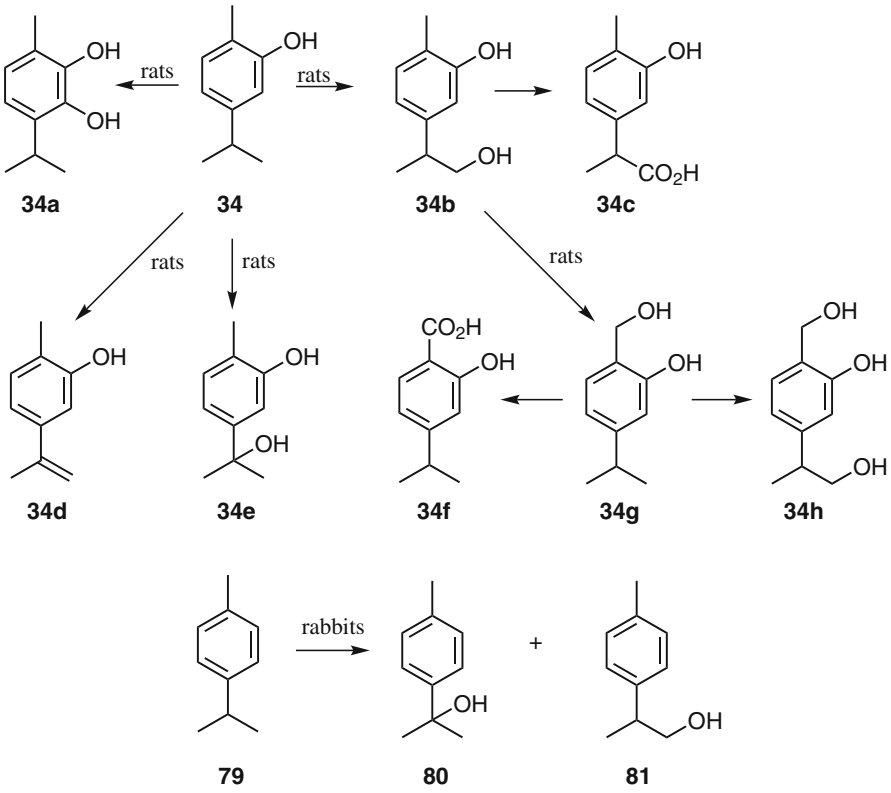
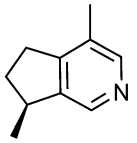


Fig. 10 Biotransformation of carvacrol (**34**) by rats and *p*-cymene (**79**) by rabbits (Noma and Asakawa 2010a, b)

vegetable, and beverage. Dietary monoterpenoids show not only antimicrobial activity but also much different activity against our body, especially antioxidant, antiinflammatory, anticancer, antiseptic, antihypertension, hypoglycemic, hypocholesterolemic, sedative, and carminative effects as seen in many foods, vegetables, and beverage to maintain our body comfortably and healthfully. Many monoterpenoids have been tested against cancer cell lines and the other human organisms, and each pure monoterpenoid possesses anticancer properties and aforementioned pharmacological activity in relatively low concentration as shown in Table 2. It is absolutely difficult for the author to gather all of foods, vegetables, processed foods, and beverages including herbal tea and alcoholic beverages from the world and to tabulate the distribution of monoterpenoids and their benefits, uses, and pharmacological activity. However, the author believes that these tables are useful for future dietary monoterpene research and hope that many significant and valuable effects for our body will be found in the known and

unknown edible plants, fungi, bryophytes, lichen, and algae and a lot of safe and healthy supplements and food additives as well as processed foods will be created.



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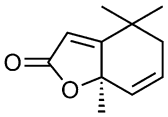

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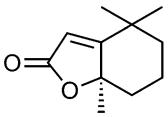

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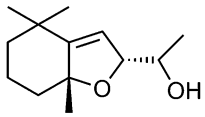

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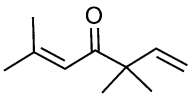

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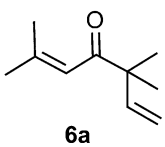

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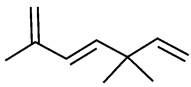

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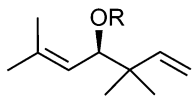

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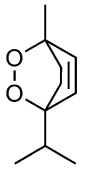

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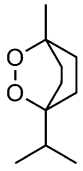

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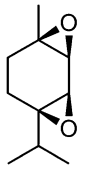
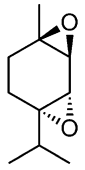
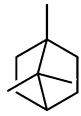


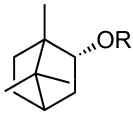
11a



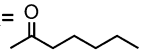
11b

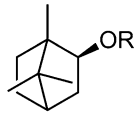


12

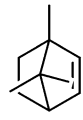


13: R=H
14: R= 
15: R= Ac
16a: R= 
16b: R= 

16c: R= 
17: R= 
18: R= 



19a: R=H
19b: R= 
20: R=Ac
21: R= 



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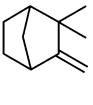

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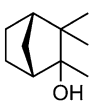

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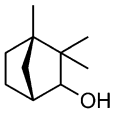

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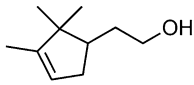

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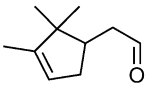
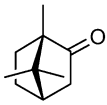


28



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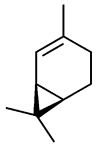

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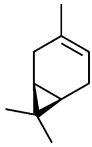
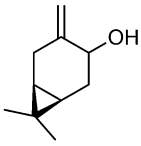
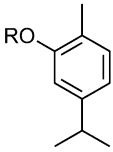


31

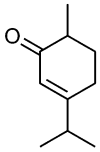
32



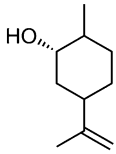
33



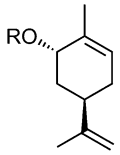
34: R=H
35: R=Me



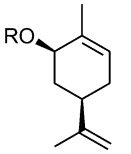
36



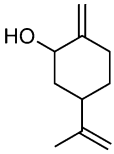
37



38a: R=H
38b: R=Ac



39a: R=H
39b: R=Ac



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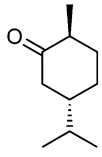

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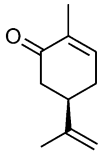

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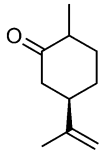

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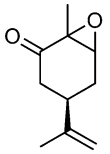
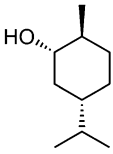


44



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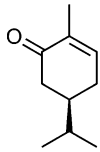

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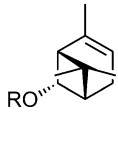
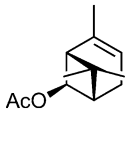


47: R=H
48: R=Ac



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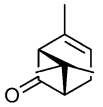

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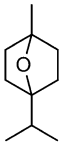
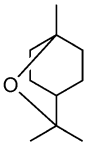
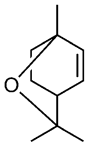


51



52



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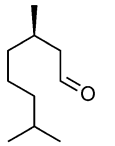

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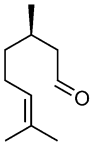

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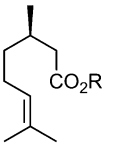
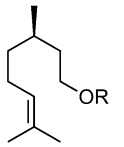
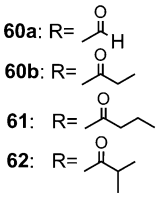


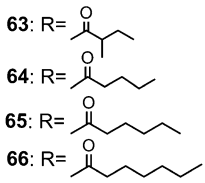
56: R=H
57: R=Me



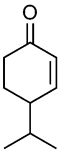
58: R=H
59: R=Ac



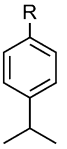
60a: R=
60b: R=
61: R=
62: R=



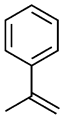
63: R=
64: R=
65: R=
66: R=



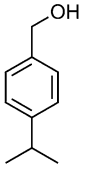
67



68a: R=H
68b: R=OH



69



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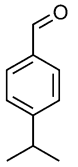

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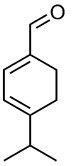
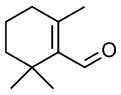
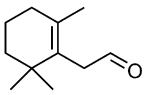


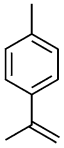
72



73



74



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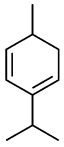
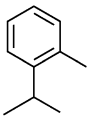


76



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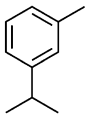

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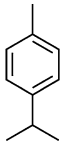

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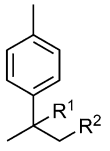
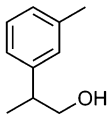
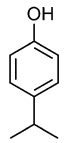


80: R^1=OH, R^2=H
81: R^1=H, R^2=OH



82



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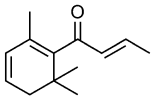

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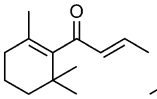

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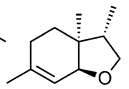

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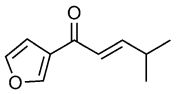
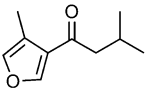
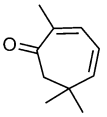


87a



87b



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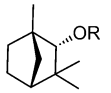
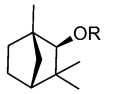
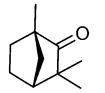
88



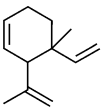
89: R=H
90: R=Ac



91: R=H
92a: R=Ac



92b



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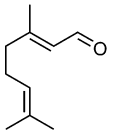

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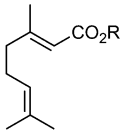
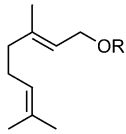
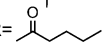
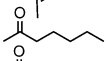
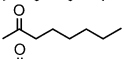
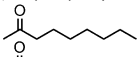
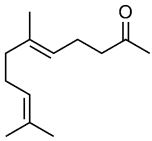


95: R=H
96: R=Me



97: R= H
98: R= 
99: R= Ac
100: R= 
101: R= 
102: R= 
103: R= 
104: R= 

105: R= 
106: R= 
107: R= 
108: R= 
109: R= 
110: R= 



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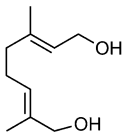

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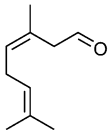

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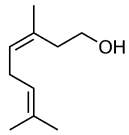

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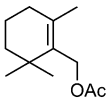

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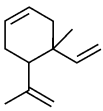

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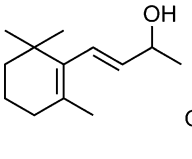

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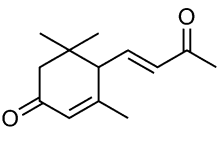

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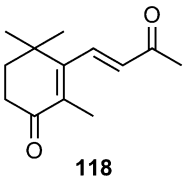

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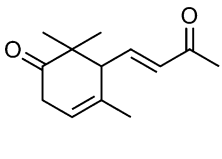

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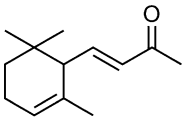

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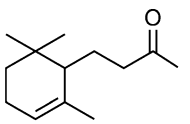

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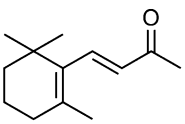

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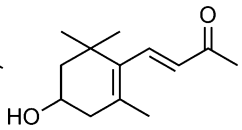

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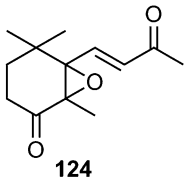

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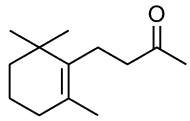

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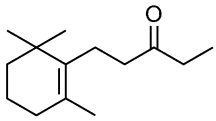

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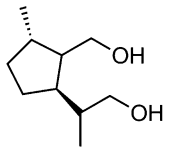

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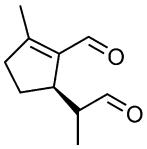

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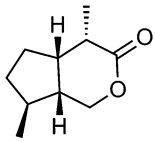

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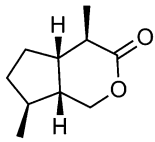

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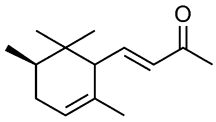

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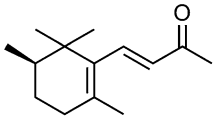
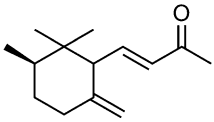
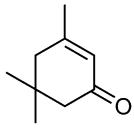


130b



130c



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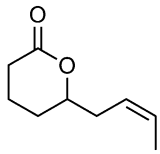
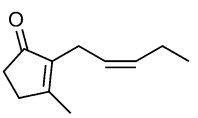
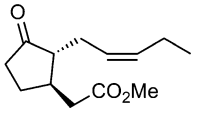


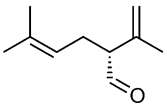
132



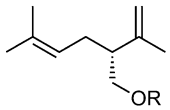
133



134



135



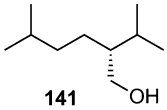
136: R=H

137: R=Ac

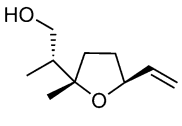
138: R=

139: R=

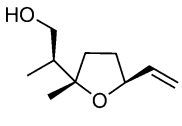
140: R=



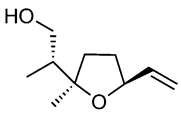
141



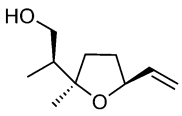
142



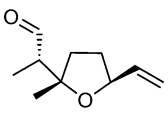
143



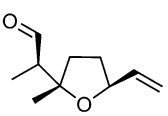
144



145



146



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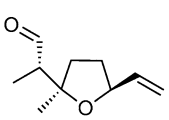
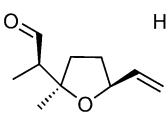
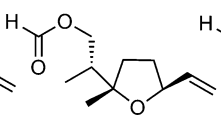


148



149



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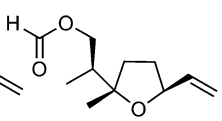
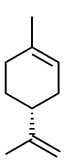
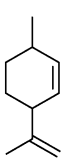


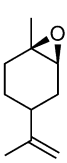
151



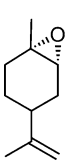
152



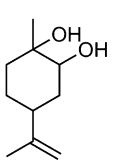
153



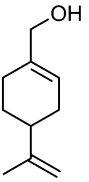
154a



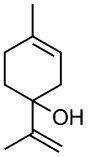
154b



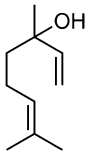
155



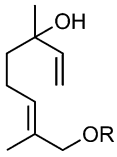
156



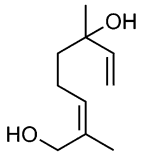
157



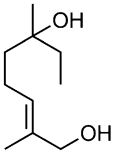
158



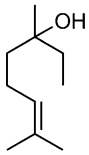
159a: R=H
160: R=Ac



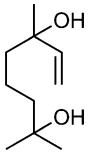
159b



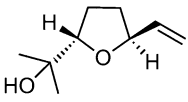
161



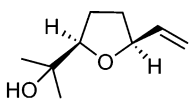
162



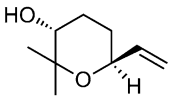
163



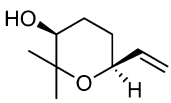
164a



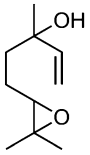
164b



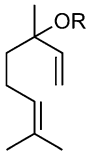
165a



165b



166

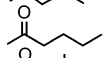


167: R= 

168: R=Ac

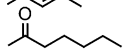
169: R= 

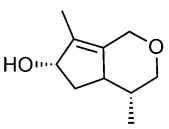
170: R= 

171: R= 

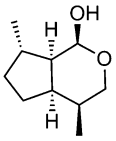
172: R= 

173: R= 

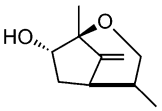
174: R= 



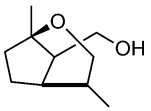
175a



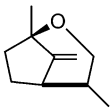
175b



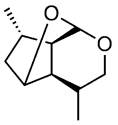
176



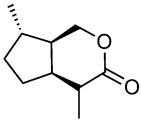
177



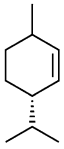
178



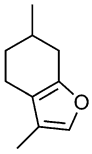
179



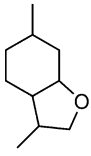
180



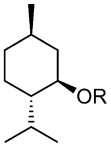
181



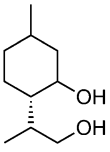
182a



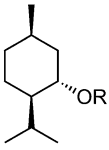
182b



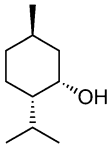
183: R=H
184: R=Ac



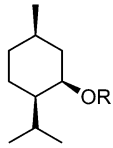
185



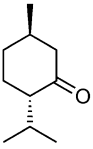
186a: R=H
186b: R=Ac



187



188a: R=H
188b: R=Ac



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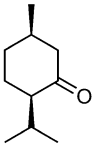

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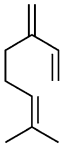
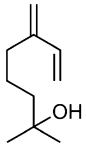
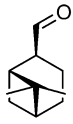


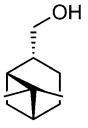
191



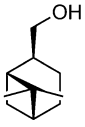
192



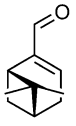
193



194a

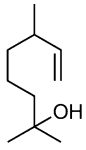


194b

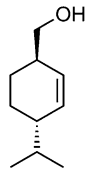


195

196: R=H
197: R=Ac
198: R=



199



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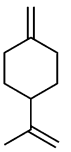

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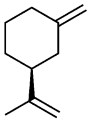
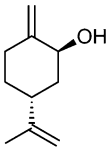
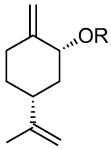


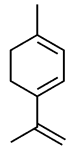
202



203a



203b: R=H
203c: R=Ac



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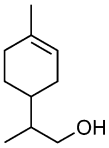

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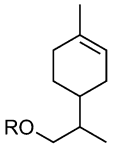
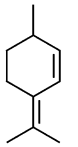
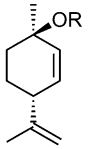


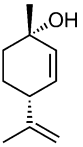
206: R=H
207: R=Ac



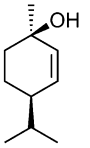
208



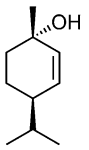
209a: R=H
210: R=Ac



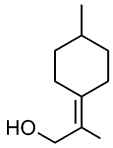
209b



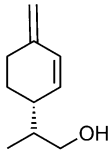
211a



211b



212



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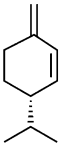
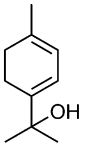
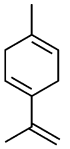


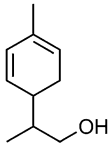
214



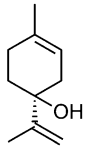
215



216



217



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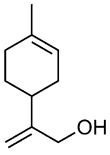
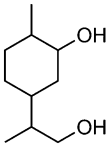


218b



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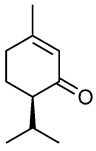

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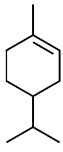
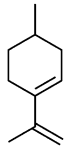
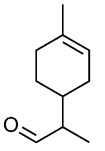


221a



221b



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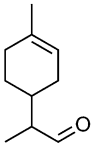

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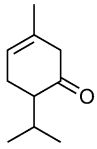

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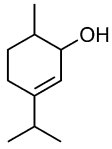
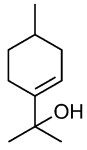
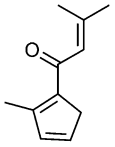


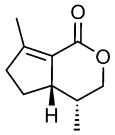
222



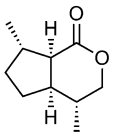
223



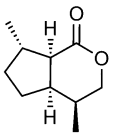
224



225



226



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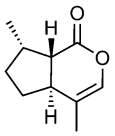
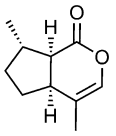
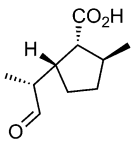


228



229



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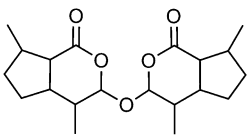

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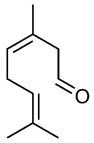
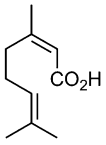
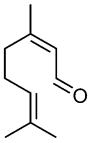


232



233



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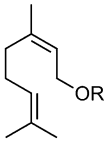


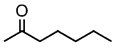
234b: R=H

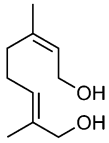
235: R= 

236: R=Ac

237a: R= 

237b: R= 

238: R= 



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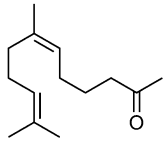

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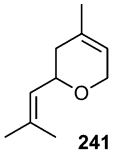

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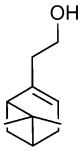

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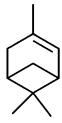

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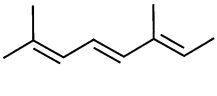

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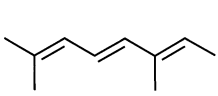

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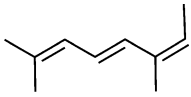

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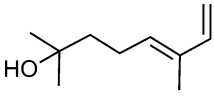

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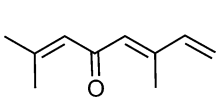

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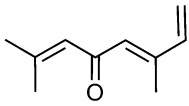

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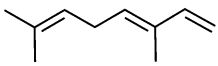

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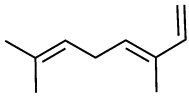

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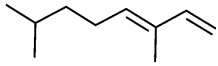

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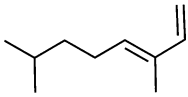

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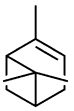

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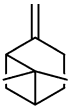

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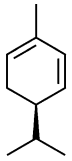

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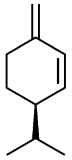

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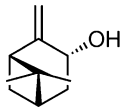
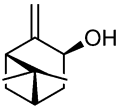


258a: R=H
259: R=Ac



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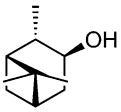

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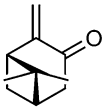

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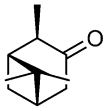

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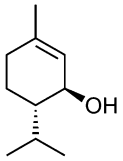

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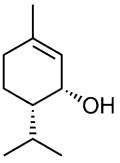
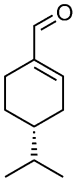


263: R=H
264: R=Ac



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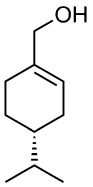

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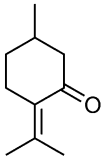

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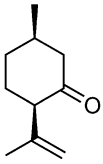

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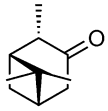

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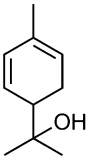

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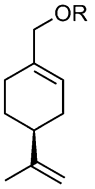
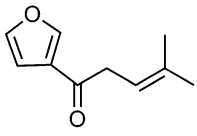


271: R=H
272: R=Ac



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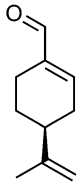

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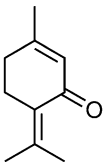

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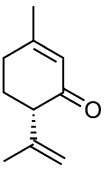

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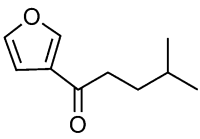

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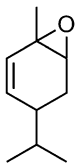

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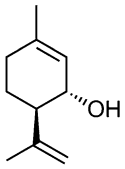

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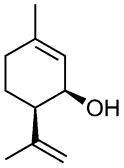

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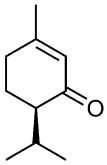

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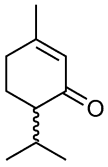

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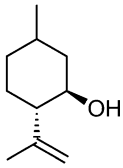

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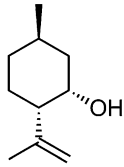

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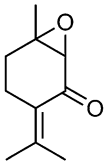

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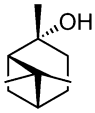

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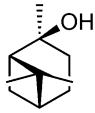

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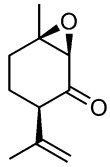

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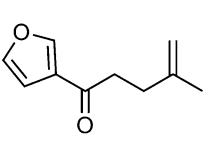

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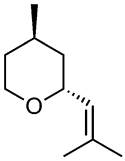

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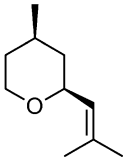

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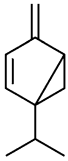

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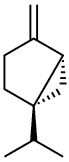
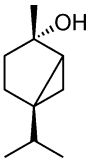
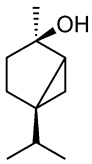


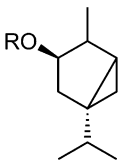
295



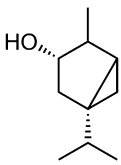
296



297

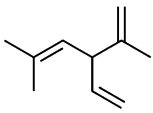


298: R=H
299: R=Ac

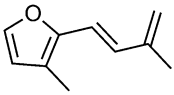


300

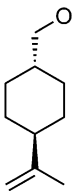
301a



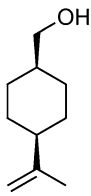
301b



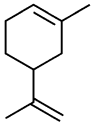
302



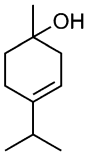
303



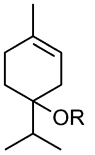
304



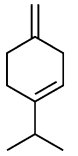
305



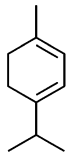
306



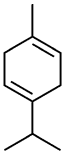
307a: R=H
307b: R=Ac



307c



308



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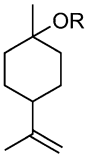
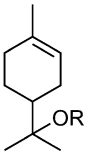
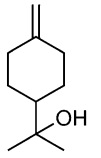


310: R=H
311: R=Ac



312: R=H
313: R=Ac
314: R=



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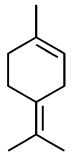

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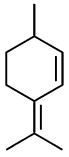

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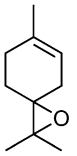

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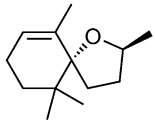

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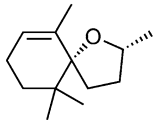

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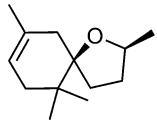

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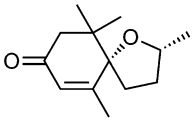
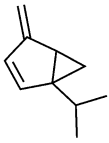
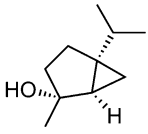


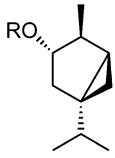
322



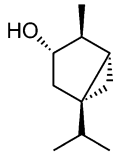
323



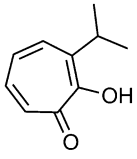
324



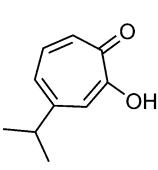
325a: R=H
325b: R=Ac



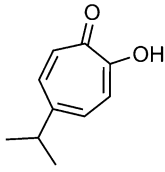
326



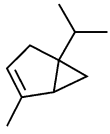
327



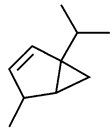
328a



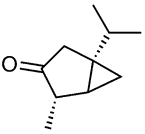
328b



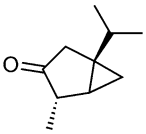
329



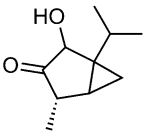
330



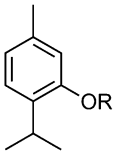
331a



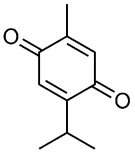
331b



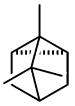
332



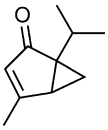
333: R=H
334: R=Me



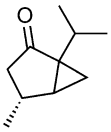
335



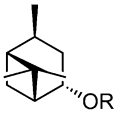
336



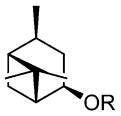
337



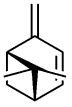
338



339a: R=H
339b: R=Ac



340a: R=H
340b: R=Ac



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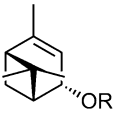
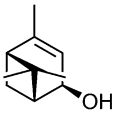


342a: R=H
342b: R=Ac



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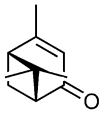

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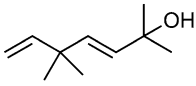

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The Names of Dietary Monoterpenpids and -Their Structure Numbers

A:

Actinidin	(**1**)
Actinidiolide	(**2**)
Actinidiolide (Dihydro-)	(**3**)
Actinidol	(**4**)
Artemisia ketone	(**5**)
Artemisia ketone (*Iso*-)	(**6a**)
Artemisia triene	(**6b**)
Artemisyl acetate	(**7**)
Artemisia alcohol	(**8**)
Ascaridole	(**9**)
Ascaridole (Dihydro-)	(**10**)
Ascaridole (*E-Iso*-)	(**11a**)
Ascaridole (*Z-Iso*-)	(**11b**)

B:

Bornane	(**12**)
Borneol	(**13**)
Bornyl formate	(**14**)
Bornyl acetate	(**15**)
Bornyl propanoate	(**16a**)
Bornyl iso-butyrate	(**16b**)
Bornyl angelate	(**16c**)
Bornyl isovalerate	(**17**)
Bornyl hexanoate	(**18**)
Borneol (*Iso*-)	(**19a**)
Bornyl formate (*Iso*-)	(**19b**)
Bornyl acetate (*Iso*-)	(**20**)
Bornyl isovalerate (*Iso*-)	(**21**)
Bornylene	(**22**)

C:

Camphene	(**23**)
Camphene hydrate	(**24**)
Camphenillol (3-Methyl-)	(**25**)
Camphenilone	(**26**)
Campholenol (α-)	(**27**)
Campholenal (α-)	(**28**)
Camphor	(**29**)
Carene (Δ^2-)	(**30**)
Carene (Δ^3)	(**31**)
Carene epoxide (Δ^2-)	(**32**)
Carene-4-ol [3(10)-]	(**33**)
Carvacrol	(**34**)
Carvacrol methyl ether	(**35**)
Carvenone	(**36**)
Carveol (Dihydro-)	(**37**)
Carveol (*E*-)	(**38a**)
Carveyl acetate *(E*-)	(**38b**)
Carveol (*Z*-)	(**39a**)
Carveyl acetate (Z-)	(**39b**)
Carveol *(Iso*-)	(**40**)
Carvomenthone	(**41**)
Carvone	(**42**)
Carvone (Dihydro-)	(**43**)
Carvone oxide	(**44**)
Carvotanacetol	(**45**)
Carvotanacetone	(**46**)
Chrysanthenol	(**47**)
Chrysanthenyl acetate (*E*-)	(**48**)
Chrysanthenyl acetate (*Z*-)	(**49**)

Chrysanthenone (*E*-) (**50**)
Cineole (1,4-) (**51**)
Cineole (1,8-) (**52**)
Cineole (2,3-Dehydro-1,8-) (**53**)
Citral [(Geraniol (**94**) & Nerol (**234b**)]
Citral (*E-iso*-) [(*Iso*-geranial (**113a**) & *Iso*-neral (**232**)]
Citronellal (Dihydro-) (**54**)
Citronellal (β-) (**55**)
Citronellic acid (**56**)
Citronellate (Methyl) (**57**)
Citronellol (**58**)
Citronellyl acetate (**59**)
Citronellyl formate (**60a**)
Citronellyl propanoate (**60b**)
Citronellyl butanoate (**61**)
Citronellyl isobutyrate (**62**)
Citronellyl 2-methylbutyrate (**63**)
Citronellyl pentanoate (**64**)
Citronellyl hexanoate (**65**)
Citronellyl heptanoate (**66**)
Cryptone (**67**)
Cumene (**68a**)
Cumenol (**68b**)
Cumene (Dehydro-) (**69**)
Cumin alcohol (**70**)
(= *p*-cymen-7-ol)
Cumin aldehyde (**71**)
Cumin aldehyde (Dihydro-) (**72**)
Cyclocitral (β-) (**73**)
Cyclocitral (β-Homo-) (**74**)
Cymene (*p*-Dehydro-) (**75**)
Cymene (*p*-Dihydro-) (**76**)
Cymene (*o*-) (**77**)
Cymene (*m*-) (**78**)
Cymene (*p*-) (**79**)
Cymene-7-ol (**70**)
(= Cumin alcohol)
Cymen-8-ol (*p*-) (**80**)
Cymen-9-ol (*p*-) (**81**)
Cymen-9-ol (*m*-) (**82**)
Cymenol (*p*-) (**83**)

D:

Damascenone(β-) (**84**)
Damascone (**85**)
Dill ether (**86**)

E:
Egoma ketone (*Iso*-) (**87a**)
Elsholtzia ketone (**87b**)
Eucarvone (**87c**)
F:
Fenchene (α-) (**88**)
Fenchyl alcohol (α-) (**89**)
Fenchyl acetate (α-) (**90**)
Fenchyl alcohol (β-) (**91**)
Fenchyl acetate (β-) (**92a**)
Fenchone (**92b**)
G:
Geijerene (**93**)
Geranial (**94**)
Geranic acid (**95**)
Geranate (Methyl) (**96**)
Geraniol (**97**)
Geranyl formate (**98**)
Geranyl acetate (**99**)
Geranyl propanoate (**100**)
Geranyl butanoate (**101**)
Geranyl iso-butanoate (**102**)
Geranyl pentanoate (**103**)
Geranyl isovalerate (**104**)
Geranyl 2-methylbutanoate (**105**)
Geranyl tiglate (**106**)
Geranyl hexanoate (**107**)
Geranyl heptanoate (**108**)
Geranyl octanoate (**109**)
Geranyl benzoate (**110**)
Geranyl acetone (**111**)
Geraniol (Hydroxy-) (**112**)
Geranial (*Iso*-) (**113a**)
Geraniol (β-*Iso*-) (**113b**)
Geranyl acetate (α-Cyclo-) (**114**)
Geyrene (**115**)
I:
Ionol (β-) (**116**)
Ionone (3-Oxo-α-) (**117**)
Ionone (3-Oxo-β-) (**118**)
Ionone (4-Oxo-α-) (**119**)
Ionone (α-) (**120**)
Ionone (α-Dihydro-) (**121**)
Ionone (β-) (**122**)

Ionone (β-4-Hydroxy-) (**123**)
Ionone (β-5,6-Epoxide) (**124**)
Ionone (β-Dihydro-) (**125**)
Ionone (δ-Dihydro-β-) (**126**)
Iridodiol (Dehydro-) (**127a**)
Iridodial (Dehydro-) (**127b**)
Iridomyrmecin (**128**)
Iridomyrmecin (*Iso*-) (**129**)
Iron (α-) (**130a**)
Iron (β-) (**130b**)
Iron (γ-) (**130c**)
Isophorone (**131**)

J:

Jasmine lactone (**132**)
Jasmone (*Z*-) (**133**)
Jasmonate (Methyl) (**134**)

L:

Lavandulal (**135**)
Lavandulol (**136**)
Lavandulyl acetate (**137**)
Lavandulyl pentanoate (**138**)
Lavandulyl 2-methylbutanoate (**139**)
Lavandulyl tiglate (**140**)
Lavandulol (Tetrahydro-) (**141**)
Lilac alcohol a (**142**)
Lilac alcohol b (**143**)
Lilac alcohol c (**144**)
Lilac alcohol d (**145**)
Lilac aldehyde 1 (**146**)
Lilac aldehyde 2 (**147**)
Lilac aldehyde 3 (**148**)
Lilac aldehyde 4 (**149**)
Lilac alcohol formate 1 (**150**)
Lilac alcohol formate 2 (**151**)
Limonene (**152**)
Limonene (*Iso*-) (**153**)
Limonene 1,2-oxide (*E*-) (**154a**)
Limonene 1,2-oxide (*Z*-) (**154b**)
Limonene diol (**155**)
Limonene-10-ol (**156**)
Limonene-4-ol (**157**)
Linalool (**158**)
Linalool (7*E*-8-hydroxy-) (**159a**)
Linalool (7*Z*-8-Hydroxy-) (**159b**)

Linalyl 8-acetate (**160**)
Linalool (8-Hydoxydihydro-) (**161**)
Linalool (Dihydro-) (**162**)
Linalool hydrate (**163**)
Linalool oxide (*E*-: furanoid) (**164a**)
Linalool oxide (Z-: furanoid) (**164b**)
Linalool oxide (*E*-: pyranoid) (**165a**)
Linalool oxide (*Z*-: pyranoid) (**165b**)
Linalool oxide (β-) (**166**)
Linalyl formate (**167**)
Linalyl acetate (**168**)
Linalyl propanoate (**169**)
Linalyl butanoate (**170**)
Linalyl pentanoate (**171**)
Linalyl iso-valerate (**172**)
Linalyl senecioate (**173**)
Linalyl hexanoate (**174**)

M:

Matatabiol (*Allo*-) (**175a**)
Matatabiol (*Neo*-) (**175b**)
Matatabi diether (5-Hydroxy-) (**176**)
Matatabi diether (7-Hydroxy-) (**177**)
Matatabi ether (**178**)
Matatabi diether (**179**)
Matatabi lactone (**180**)
Menthene (2-) (**181**)
Menthofuran (**182a**)
p-Mentha-3,9-oxide (**182b**)
Menthol (**183**)
Menthyl acetate (**184**)
Menthol (8-Hydoxy- *neo*-) (**185**)
Menthol (*Iso*-) (**186a**)
Menthol (*Iso*-) acetate (**186b**)
Menthol (*Neo*-) (**187**)
Menthol (*Neo-iso*-) (**188a**)
Menthol (*Neo-iso*-) acetate (**188b**)
Menthone (**189**)
Menthone (Iso-) (**190**)
Myrcene (**191**)
Myrcenol (**192**)
Myrtanal (**193**)
Myrtanol (*E*-) (**194a**)
Myrtanol (Z-) (**194b**)

Myrtenal (**195**)
Myrtenol (**196**)
Myrtenyl acetate (**197**)
Myrtenyl isovalerate (**198**)
Myrtenol (Dihydro-) (**199**)
p-Menth-2-en-7-ol (*E*-) (**200**)
p-mentha-1(7),8-diene (**201**)
m-Mentha-1(7),8-diene (**202**)
p-Mentha-1(7),8-dien-2-ol (*E*-) (**203a**)
p-Mentha-1(7),8-dien-2-ol (*Z*-) (**203b**)
p-Mentha-1(7),8-dien-2-yl-acetate (*Z*-) (**203c**)
p-Mentha-1,3,8-triene (**204**)
p-Menth-1-en-8-ol (**205**)
p-Menth-1-en-9-ol (**206**)
p-Menth-1-en-9-yl acetate (**207**)
p-Mentha-2,4(8)-diene (**208**)
p-Mentha-2,8-dien-1-ol *(E*-) (**209a**)
p-Mentha-2,8-dien-1-ol (*Z*-) (**209b**)
p-Mentha-2,8-dien-1-yl acetate (**210**)
p-Menth-2-en-1-ol (*E*-) (**211a**)
p-Menth-2-en-1-ol (*Z*-) (**211b**)
p-Menth-4(8)-en-9-ol (**212**)
p-Mentha-1(7),2-dien-8-ol (**213**)
p-Mentha-1(7),2-diene (**214**)
p-Mentha-1,3-dien-7-ol (**215**)
p-Mentha-1,4,8-triene (**216**)
p-Mentha-1,5-dien-9-ol (**217**)
p-Mentha-1,8-dien-4-ol (**218a**)
p-Mentha-1,8-dien-9-ol (**218b**)
p-Mentha-2,8-diol (*E*-) (**219**)
p-Menth-1-en-3-one (**220**)
p-Menth-1-ene (**221a**)
p-Mentha-3,8-diene (**221b**)
p-Menth-1-en-8-al (**221c**)
p-Menth-1-en-9-al (**221d**)
p-Menth-6-en-3-one (**221e**)
p-Menth-3-en-2-ol (**222**)
p-Menth-3-en-7-ol (**223**)

N:

Naginata ketone (**224**)
Nepetalactone(*Neo*-) (**225**)
Nepetalactone (Dihydro-) (**226**)

Nepetalactone (*Iso*-dihydro-) (**227**)
Nepetalactone (*E,Z*-) (**228**)
Nepetalactone (*Z,E*) (**229**)
Nepetalic acid (**230**)
Nepetalic anhydride (**231**)
Neral (*Iso*-) (**232**)
Neric acid (**233**)
Neral (**234a**)
Nerol (**234b**)
Neryl formate (**235**)
Neryl acetate (**236**)
Neryl *iso*-butanoate (**237a**)
Neryl *iso*-valerate (**237b**)
Neryl hexanoate (**238**)
Nerol (Hydroxy-) (**239**)
Neryl acetone (**240**)
Neryl oxide (**241**)
Nopol (**242**)
Norpinene (3,6,6-Trimethyl-2-) (**243**)

O:

Ocimene (*Neo-allo*-) (**244**)
Ocimene (*E-allo*-) (**245**)
Ocimene (*Z-allo*-) (**246**)
Ocimenol (*Z*-β-) (**247**)
Ocimenone (*E*-β-) (**248**)
Ocimenone (*Z*-β-) (**249**)
Ocimene (*E*-β-) (**250**)
Ocimene (Z-β-) (**251**)
Ocimene (*E*-dihydro-) (**252**)
Ocimene (*Z*-dihydro-) (**253**)

P:

Pinene (α-) (**254**)
Pinene (β-) (**255**)
Phellandrene (α-) (**256**)
Phellandrene (b-) (**257**)
Pinocarveol (*E*-) (**258a**)
Pinocarveol (*Z*-) (**258b**)
Pinocampheol (=3-pinanol) (**258c**)
Pinocarveyl acetate (**259**)
Pinocarvone (**260**)
Pinocamphone (*E*-) (**261**)
Piperitol (*E*-) (**262**)

Piperitol (*Z*-) (**263**)
Piperityl acetate (**264**)
Phellandral (α-) (**265**)
Phellandrol (**266**)
Pulegone (*Z*-) (**267**)
Pulegone (*Z-iso*-) (**268**)
Pinocamphone (*Iso*-) (**269**)
Phellandren-8-ol (α-) (**270**)
Perilla alcohol (**271**)
Perillyl acetate (**272**)
Perilla ketone (**273**)
Perillaldehyde (**274**)
Piperitenone (**275**)
Piperitenone (*Iso*-) (**276**)
Perillene (**277**)
Phellandrene oxide (**278**)
Piperitenol (*E- iso*-) (**279**)
Piperitenol (*Z-iso*-) (**280**)
Piperitone (**281**)
Piperitone (*Iso*-) (**282**)
Pulegol (*Iso-)* (**283**)
Pulegol (*Neo-iso*-) (**284**)
Piperitenone oxide (*Z*-) (**285**)
Pinene hydrate (*E*-) (**286**)
Pinene hydrate (*Z*-) (**287**)
Pinene oxide (β-) (**288**)
Piperitone oxide (Z-) (**289**)
Perilla ketone (*Iso*-) (**290**)

R:

Rose oxide (*E*-) (**291**)
Rose oxide (*Z*-) (**292**)

S:

Sabinene (Dehydro-) (**293**)
Sabinene (**294** & **295**)
Sabinene hydrate (*E*-) (**296**)
Sabinene hydrate (Z-) (**297**)
Sabinol (*E*-) (**298**)
Sabinyl acetate (*E*-) (**299**)
Sabinol (*Z*-) (**300**)
Santene (**301a**)
Santolina triene (**301b**)
Shisofuran (**302**)

Shisool (*E*-) (**303**)
Shisool (*Z*-) (**304**)
Sylvestrene (**305**)

T:

Terpinen-1-ol (**306**)
Terpinen-4-ol (**307a**)
Terpinen-4-yl acetate (**307b**)
Terpinene(β-) (**307c**)
Terpinene (α-) (**308**)
Terpinene (γ-) (**309**)
Terpineol (*Z*-β-) (**310**)
Terpinyl acetate (*Z*-β-) (**311**)
Terpineol (α-) (**312**)
Terpinyl acetate (α-) (**313**)
Terpinyl propanoate (α-) (**314a**)
Terpinyl pentanoate (α-) (**314b**)
Terpineol (δ-) (**315**)
Terpinolene (**316**)
Terpinolene (*Iso*-) (**317**)
Terpinolene-4,8-oxide (**318**)
Theaspirane (**319**)
Theaspirane-A (**320**)
Theaspirane-B (**321**)
Theaspirone (**322**)
Thuja-2,4(10)-diene (**323**)
Thujanol (*Z*-4-) (**324**)
Thujanol (*Neo*-3-) (**325a**)
Thujanol acetate (*Neo*-3-) (**325b**)
Thujyl alcohol (**326**)
Thujapricin (α-) (**327**)
Thujapricin (β-) (**328a**)
Thujapricin (γ-) (**328b**)
Thujene (α-) (**329**)
Thujene (β-) (**330**)
Thujone (α-) (**331a**)
Thujone (β-) (**331b**)
Thujone-2-ol (3-) (**332**)
Thymol (**333**)
Thymol methyl ether (**334**)
Thymoquinone (**335**)
Tricyclene (**336**)

U:

Umbellulone (**337**)
Umbellulone (Dihydro-) (**338**)

V:	
Verbanol (*E*-)	(**339a**)
Verbenyl acetate (*E*-)	(**339b**)
Verbanol (*Iso*-)	(**340a**)
Verbanyl acetate (*Iso*-)	(**340b**)
Verbenene	(**341**)
Verbenol (*E*-)	(**342a**)
Verbenyl acetate (*E*-)	(**342b**)
Verbenol (*Z*-)	(**343**)
(=2-pinen-4-ol)	
Verbenone	(**344**)
Y:	
Yomogi alcohol	(**345**)

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Dietary Diterpenoids

17

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Contents

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Abstract

A number of foods, vegetables, fruits, and beverages (tea, coffee, and liqueur) contain many kinds of diterpenoids (mainly abietanes, cembranes, clerodanes, cyatanes, dolabellanes, fusicoccanes, kauranes, labdanes, pimaranes, taxanes, trachilobanes, totaranes) and their related compounds. This chapter summarizes the distribution of diterpenoids in dietary foods, vegetables, fruits, beverages, and processed foods, and their biological and pharmacological activity, effects, and application to our health, as well as metabolic pathways of several diterpenoids, which are widely distributed in dietary foods, by microorganisms and mammals.

Keywords

Diterpenoids · Coffee · *Ginkgo* · *Salvia* · *Stevia* · Biological activity · Biotransformation · Benefits

17.1 Introduction

In China, Korea, and Japan, a great number of higher and spore forming plants as well as mushrooms have been used as foods, including "functional foods," vegetables, fruits, and beverages as tea, coffee, and alcoholic drinks for human good health. In South-east and central Asia and Africa and South America, many different plants species from Europe and far east have been used as the same purpose as those in the above mentioned regions. Mediterranean region, the Aegean coast of Turkey, Greece and Balkan Peninsula as well as the central Asia, India, and Indonesia have been known as the origin of ancient medicines. Especially Greece and Turkey, the culmination of the knowledge in medicinal herbs and aromatic plants, morphology and anatomy have been developed.

In Mediterranean region, there are a great number of medicinal plants belonging to the Lamiaceae, for example, *Baccharis, Nepeta, Salvia*, *Stachys*, *Sideritis, Teineum*, and *Thymus* including diterpenoids (Carovic-Stanko et al. 2016). Madeira and Porto Santo Islands are ethnopharmacologically interesting because medicinal plants are recognized and many of which contain diterpenoids have been used as the herbal tea, infusion, or decoction used as antiasthmatic, antidiabetic, antirheumatic, antihypertensive, diuretic, insecticide, antibacterial, antifungal, antioxidant, sedative, emmenagogue or relieving coughs and fever, and many other diseases (Rivera and Obon 1995).

In this chapter, the distribution of diterpenoids and their related compounds in the representative foods, fruits, vegetables, and beverages including medicinal herbs, their biological and pharmacological activity and metabolism in mammalian body and benefits are summarized. Coffees, *Stevia*, *Ginkgo*, and *Zinger* species are focused as the dietary foods, food additives, beverage, and medicinal drugs in detail.

17.2 Distribution of Diterpenoids and Related Compounds in Foods Vegetables, Fruits, and Beverages

The diterpenoids are one of the most important secondary metabolites, widely distributed not only in terrestrial plants but also in marine organisms. Generally diterpenoids don't possess odorous properties, however, many diterpenoids indicate very bitter and pungent and some of which show potent sweet taste.

They are derived from geranylgeranyl pyrophosphate and are classified into several types, mainly abietanes, atisanes, bayeranes, cassanes, cembranes, clerodanes, cyatanes, daphnanes, dolabellanes, dolastanes, fusicoccanes, gibberellanes, halimanes, ingenanes, kauranes, labdanes, pimaranes, podocarpanes, rosanes, stemodanes,

stemaranes, taxanes, tiglicanes, trachilobanes, among which abietanes, clerodanes, kauranes, labdanes, and gibberellanes are the most popular and widely distributed as dietary phytochemicals.

In Charts 1 and 2, the most abundant diterpene skeletons found in the foods and beverages are shown.

Many abietanes contain an aromatic moiety in C-ring and they are widely distributed in terrestrial medicinal beverage plants especially in *Salvia* species and *Coffea* (Coffees). Cembranes, dolabellanes, and dolastanes have been mainly found in marine organisms, such as brown algae, *Dictyota* species, and even terrestrial plants, like some bryophytes. Taxanes have been isolated from almost only *Taxus* species, and paclitaxel (=taxol) (**1**) is now been used as clinically an important anticancer drug. Some fungi and liverworts produce cyatane diterpenoids. *Gibbellera fusikroii* mainly elaborates gibbellane diterpenoids. *Podocarpu*s species biosynthesizes podocarpanes as predominant metabolites. Connolly and Hill (1991) published the encyclopedia of di-, sester-, and triterpenoids in which more than 1000 of the structures of diterpenoids have been presented.

Hanson et al. (2017) reported the review article which covered the isolation and structure elucidation of terrestrial origin diterpenoids that include abietanes, cembranes, kauranes, labdanes, and their cyclic products including 228 references.

Li et al. (2016) reviewed naturally occurring clerodane diterpenoids with 533 references and 1317 structures, and their biological and pharmacological activity. The distribution of diterpenoids with typical biological and pharmacological properties was reviewed by Vasas and Hohman (2014) and Remy and Litaudon (2019). Islam et al. (2016) obtained plant-derived diterpenoids data from Science Direct, PubMed, Web of Science, Sopus, and various databases and described a total of 119 medicinal plants containing diterpenoids and their pharmacological activities (anticancer, antidiabetic, antifungal, antimicrobial, anti-inflammatory, antioxidant, antiviral, cardio-, chemo-, gastro-, hepato-, immune-, and neuroprotective, cytotoxic, genotoxic, and mutagenic with 232 references reported between 2005 and 2015).

A number of daphnane type diterpenoids have been found in species belonging to the Euphorbiacae and Thymelaceae families. Jin et al. (2019) reported a review article of structures of daphnanes, and their biological and pharmacological activities with 41 references. Appendino (2016) reported the distribution of ingenane type diterpenoids which are derived from daphnanes as seen in Chart 3, and their bioactivity with 221 references.

In Table 1, a number of different kinds of foods, fruits, vegetables, and beverages have been alphabetically listed, and the distribution of diterpenoids and their related compounds as well as their biological and pharmacological effects and benefits have also been briefly described. Up to now more than 3000 diterpenoids have been found in plants and mushrooms, and these constitute acyclic (phytane), monocyclic, bicyclic, tricyclic, and tetracyclic diterpenoids. They occur in nature widely as hydrocarbons, alcohols, ketones, and lactones. There are also many kinds of carboxylic diterpenoids at C-18 or C-19 (1,1-dimethyl group at C4 in A-ring) of abietanes, clerodanes, kauranes, and labdanes as well as their methyl and higher

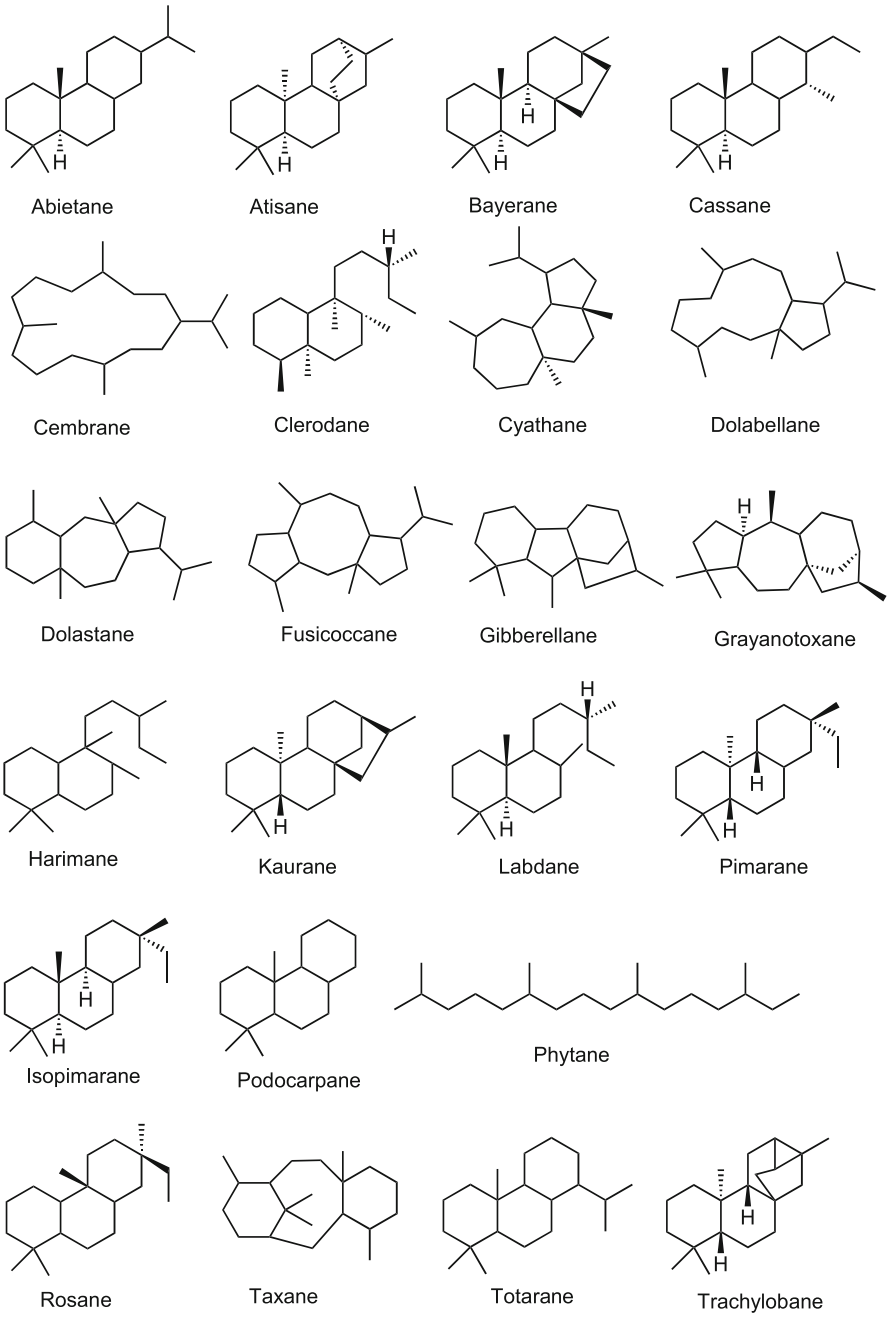


Chart 1 Main diterpene skeletons found in foods, fruits, vegetables, medicinal plants, and beverages

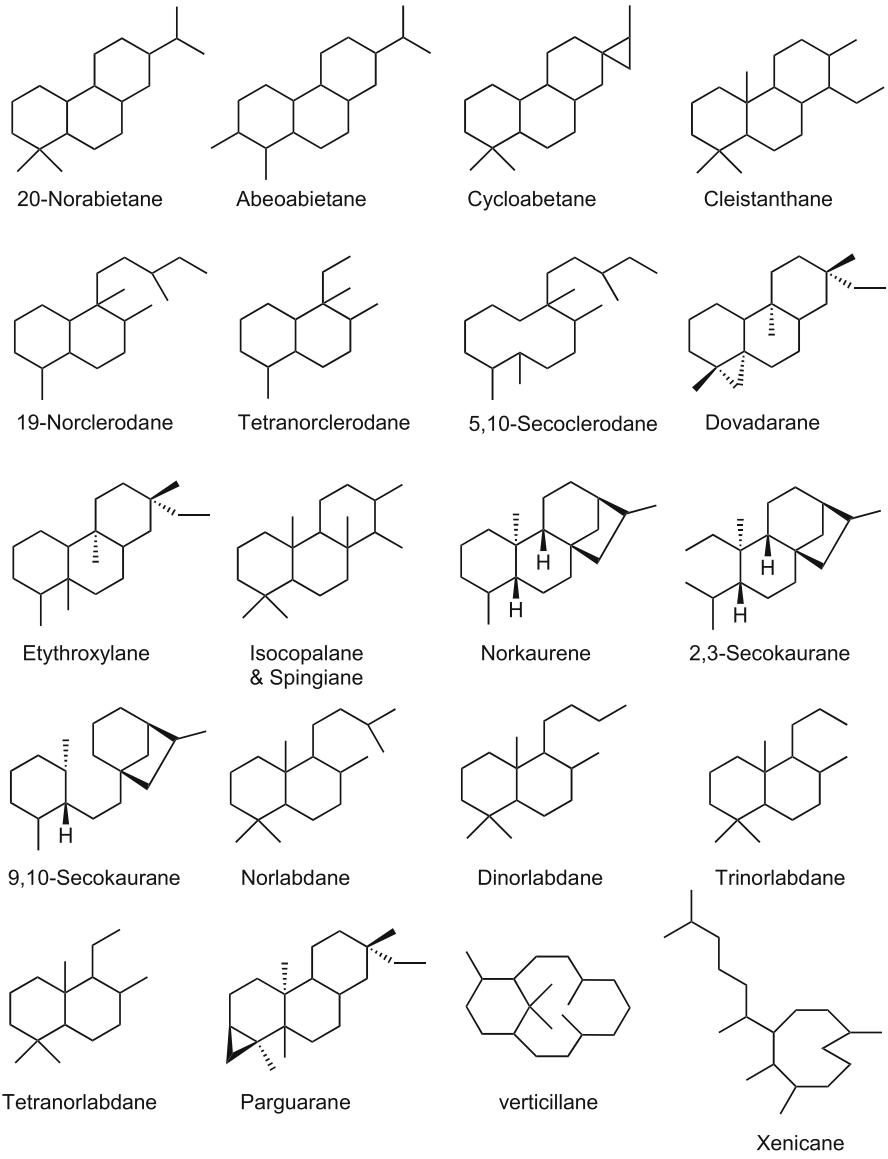


Chart 2 Minor diterpene skeletons found in foods, fruits, vegetables, medicinal plants, and beverages

fatty acid esters are also isolated from or confirmed in the dietary. One of the most characteristic features of naturally occurring diterpenoids is that a great number of furan and γ-lactone rings containing diterpenoids have been isolated and their stereochemistry elucidated using a combination of modern nuclear magnetic resonance (NMR), mass spectrum (MS), circular dichroism (CD) and X-ray

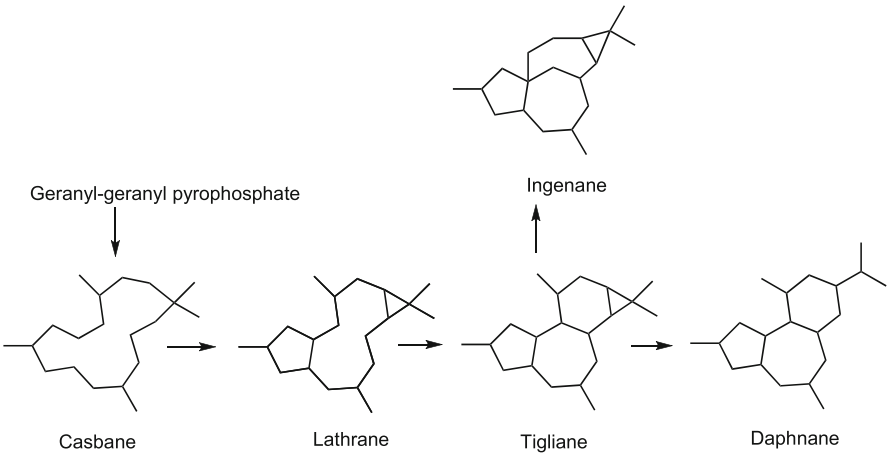


Chart 3 Diterpenpoid (casbane, latharane, tigriane, daphnane, and ingenane) skeletons found in foods, fruits, vegetables, medicinal plants, and beverages

crystallographic analysis. The second significant chemical profile of diterpenoids is that a number of isolated diterpenoids, such as clerodanes, kauranes, and pimaranes, possess the enantiomeric forms, although there are several exceptions.

As seen in Table 1, each dietary food and beverage contain only specific diterpenoids; however, the chemical profile of the others are so complex, including the different skeletal diterpenoids, and they are highly oxygenated.

Here, the species names and the distribution of diterpenoids in several well-known foods, beverages, and medicinal plants are surveyed.

In the following paragraphs, several representative genus and family including dietary diterpenoids have been discussed. In Charts 6–13 and Table 1, the representative diterpenoids (**60–185**) found in various foods and medicinal plants have been presented in addition to the diterpenoids (**1–59d**) which have been discussed in each paragraph.

17.2.1 *Agathis* (Kauri or Dammara) (Araucariaceae)

The *Agathis* grows in Oceanian region, mainly in Australia and New Zealand.

There are 22 species in these regions. These species belonging to this genus is one of the most valuable and sought after timber trees in Southeast Asia and it is traded on the international market. It also provides high-quality resins among which those from *A. borneensis*, *A. dammara*, *A. lanceolata*, *A. macrophylla*, and *A. philippinensis* are the most important commercially. 3α-Hydroxy-13*S*-16- nor-pimar-7-en-15-oic acid and 13*S*-pimar-7-en-3α,15,16-triol, and kaur-16-en-3α,13-diol, kauran-3α,13,16-α-triol, and (*E*)- and (*Z*)-communic acid have been isolated from the heartwood of the Fijian species *Agathis vitiensis* together with agatharesinol, abietic acid (**2**), and agathic acid. (Z)-communic acid indicates protective effects against UVB-induced

Table 1 The dietary plants and mushroom names used as foods, beverages, and herbal medicines including diterpenoids and their related compounds and application, benefits, and effects

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Abies georgei	7-Oxocallitrisic acid	Antiplatelet aggregation, antithrombic
Abies sachalinensis	Abieta-7,17-dien-12α-methoxy-18-oic acid, dehydroabietic acid (**43**), neoabietic acid, levopimaric acid	Foods, flavor source (soap, bath additive, deodorant) Antifungal, antioxidant, α-glucose inhibitory, humectants, skin conditioning
Abies siberica (Siberian Fir)	7,13-Abietadien-18-oic acid (=abietic acid) (**2**), 7,13-abitadien-18-ol (=abietinol), abietinal, abientol, iso-abienol	Antibacterial, antifungal, antihypertensive, anti-inflammatory, antioxidant, analgesic, expectorant, stimulant For arthritis, anxiety, colds, digestion, muscle recovery, pain, flu, wound healing
Acacia leucophloea	15*R*,16-Epoxy-8(14)-pimarene-1β,11α-diol (=leucophleoxol), 8(14)-pimarene-1β,15*R*,16-triol (=leucephaleol), leucoxol	Analgesic, antipyretic, antidote for snake bite, antimicrobial, anthelmintic, antipyretic, astringent, gastrointestinal and respiratory activity, expectorant For bronchitis, cough, demulcent, dental caries, diarrhea, dysentery, infection preventive, internal and external hemorrhages, intermittent fevers, skin diseases, stomatitis, styptic, ulcers, thermogenic, vulnerary, vomiting, wound healing
Acanthopanax koreanum	Acanthoic acid, *ent*-kaur-16-en-19-oic acid (**47**), 16β,17-dihydroxy-*ent*-kauran-19-oic acid (**4**), pimara-9(11),15-dien-19-ol,pimara-9(11),15-dien-19-oic acid (**60**), 19-acetoxy-pimara-9(11), 15-diene, pimara-9(11), 15-diene, 16α*H*,17-isovaleryloxy-*ent*-kauran-19-oic acid, 16α-hydroxy-17-isovaleryloxy-*ent*-kauran-19-oic acid, paniculoside-IV,16α-hydroxy-*ent*-kauran-19-oic acid, acanthol, acanthoic acid, 7β-hydroxy-*ent*-pimar-8(14),15-dien-19-oic acid, acantholoreoic acid A, *ent*-kaur-16-en-19-oic acid (**47**), 16α-hydroxy-*ent*-kauran-19-oic acid, 16α*H*,17-isovaleryloxy-*ent*-kauran-19-oic acid, 16α,hydroxy-17-isovaleryloxy-*ent*-kauran-19-oic acid	Hepatoprotective, TNF-α secretion inhibitory, tyrosine phosphate 1B inhibitory, sedative, tonic For diabetes, rheumatism

Acremonium luzulae	7,16-Isopimaradiene-2α,3β,19-triol (=virescenoside A), virescenoside E, virescenoside F, virescenside L	Immunomodulatory, immunostimulatory
Acritopappus confertus	16-Hydroxy-*ent*-labda-7, 13-dien-15-oic acid, 16,17-dihydroxy-*ent*-labda-7,13*E*-dien-15-oic acid, 16-hydroxy-17-oxo-*ent*-labda-7,13*E*-dien-15-oic acid,17-hydroxy-16-acetoxy-*ent*-labda-7,13*E*-dien-15-oic acid, 16-hydroxy-17-methoxy-*ent*-labda-7,13(14)-dien-15-oic acid, 7β,15-dihydorxy-*ent*-labda-8(17),13(14)-dien-16-oic acid lactone	
Acritopappus morii	7β-Hydroxypolyalthine, 17-hydroxyisopolyalthine, 7,15,16-trihydroxy-*ent*-labda-7,12(14)-diene	
Acritopappus teixeirae	16-Oxo-koval-3-en-15-oic acid	
Acritopappus pushagei	16-Acetoxy-18-hydroxy-kolavenic acid, 16,18-dihydroxykalavenic acid, 16-acetoxy-18-oxo-kolavenic acid, 16-hydroxy-18-oxo-kolavenic acid, 2β,15-didihydroxy-kolavenic acid, 15-hydroxy-2-oxo-kolavenic acid, acritoppusol, 16,18-dihydroxykolavenic acid lactone, 12,15,16-trihydroxy-*ent*-labda-7,13-dien-15-oic acid lactone, 12*S*,16-dihydroxy-*ent*-labda-7,13-dien-15,16-olide, 16,18-dihydroxy-3,13*Z*-clerodadien-15-oic acid (=16,18-dihydroxykolavenic acid), 2-hydroxy-3,13-clerodadien-16,15-olide (=2,15-diydroxykolavenic acid lactone), 2-oxo-3,13-clerodadien-16,15-olide, *ent*-18-acetoxy-3,13-kolavadien-16,15-olide	Anticancer
Aframomum aulacocarpos	Aulacocarpinolide, aulacocarpin A, aulacocarpin B, 8β(17)-epoxy-14,15,16-trihydroxylabd-12*E*-ene (=aulacocarpin C) (**61**), 15,16-epoxy-14*Z*,16*Z*-dimethoxylabda-8(17), 12*E*-diene (=aulacoparpin D), aframodial	Food, spice Antifungal, antimicrobial
Aframomum deniellii	8β,17-Epoxy-12*E*-labdene-15,16-dial (=aframodial)	Antifungal, antihypercholesterolemic, cytotoxic
Agathis australis	8,(14),15-Isopimaradien-18-ol (=isopimarinol), (*Z*)-communic acid, (*E*)-communic acid (**3**), levopimaric acid, sandaracopimaric acid, abietic acid (**2**), agathic acid	For wound healing

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Agathis lanceolata	19-Nor-8(17),13*E*-labdadien-15-oic acid (=19-noranticopalic acid), (*Z*)-communic acid, (*E*)-communic acid (**3**), dehydroabietic acid (**43**), abietic acid (**2**), neoabietic acid, agathic acid, agathalic acid	Edible (seed) Antiseptic For vulnerary
Agathis macrophylla	(*Z*)-Communic acid, (*E*)-communic acid (**3**), dehydroabietic acid (**43**), abietic acid (**2**), neoabietic acid, agathic acid, agathalic acid	
Agathis microstachys	Abietic acid (**2**), agathic acid, neoabietic acid	
Agathis robusta	Abietic acid (**2**), 8(14),12-abietadien-18-oic acid (=laevopimaric acid), agathic acid, neoabietic acid, communic acid	Edible (seed)
Agathis vitiensis	3-Hydorxy-13*S*-16-nor-pimar-7-en-15-oic acid, 13*S*-pimar-7-en-3α,15,16-triol, kaur-16-en-3α, 13-diol, kauran-3α,13,16α-triol, agatharesinol, (*Z*)-communic acid, (*E*)-communic acid (**3**), sandaracopimaric acid, dehydroabietic acid (**43**), abietic acid (**2**), neoabietic acid, agathalic acid	
Agerantia ixiocladon	4-Hydroxy-13-cleroden-15-oic acid	
Ageratina saltillensis	16-Hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3α,4β,16-trihydroxy-5β,l0β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3α,4β-dihydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3α-methoxy-4β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3-oxo-4β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 2β-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 2β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-3,13(14)-diene-15,16-olide, 13*Z*-2β-hydroxy-5β,10β-*cis*-l7α,20β-cleroda-3,13(14)-diene-15-oic acid, 13*Z*-2-oxo-5β,10β-*cis*-17α,20β-cleroda-3,13(14)-diene-	For epilepsy, hypertension, mental disorders, schizophrenia, snake bite

	15-oic acid, 3,4β-epoxy-5β,10β-*cis*-17α,20α-clerodaα-l5-oic acid, 16-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-15-o1, 20-hydroxygeranylnerol, *ent*-3β,4α-dihydroxy-13-cleroden-15,16-olide, 3α,4α-dihydroxy-13-clerodanediol, *ent*-4α-hydroxy-3-oxo-13-cleroden-15,16-olide, *ent*-2α-hydroxy-3,13*Z*-clerodadien-15-oic acid, *ent*-3α,4α-epoxy-2α-hydroxy-13-cleloden-15,16-olide	
Aglaia odorata	3-Hydroperoxydolabella-4(16),7-dien-18-ol, 7,8-epoxydolabella-3-en-18-ol, 3,4-epoxydolabella-4(16),7-dien-3-one, 3,4-epoxy-dolabella-7-en-8-ol, 18-hydroxydolabella-3,7-dien, dolabella-3,4-dien-10,18-diol (**62**), dolabella-4(16),7-dien-3,18-diol, 3,4-epoxy-dolabella-7-en-18-ol, 18-hydroxydolabella-4(16),7-dien-3-one, dolabella-4(16),7,18-trien-3-ol	Anticancer, anti-leukemic, anti-HSV-1, cytotoxic (HL-60: human myeloid leukemia, SMMC-7721: hepatocellular carcinoma; A-549: lung cancer cells), insecticidal For bruises, febrifuge, heart disease, toxin by causing vomiting, traumatic injury, vomiting
Ajuga bracteosa	Ajubractin A (**63**), B-D, clerodin, 3-*epi*-caryoptin, ajugapitin, 14,15-dihydroclerodin, 3-*epi*-14,15-dihydrocaryoptin, ivain, 14,15-dihydroajugapitin, ajubratin E, 15-hydroxyajubractin, 14-hydro-15-hydroxyajugachin A, 14-hydro-15-hydroxyajugapitin, 15-*epi*-lupulin	Edible (leaves and roots) Antimicrobial, insect antifeedant
Ajuga chamaepitys	Ajugachin A, ajugachin B ajugapitin, 14,15-dihydroajugapitin, chamaepitin	Antioxidant, cytotoxic, diuretic, emmenagogue, stimulant For female disorders, gout, rheumatism
Ajuga ciliata	Neoclerodanes	Cytotoxic (SH-SY5Y: neuroblastoma cell), nitric oxide (NO) production inhibitory
Ajuga decumbens	Neoclerodanes	NO production inhibitory
Ajuga iva	Ivain I (=4,18:11,16:15,16-triepoxy-14-clerodene-2,3,6,19-tetrol), ivain II, ivain III, ivain IV	Antibacterial, anticancer, antidiabetes, antifeedant, antimicrobial, antioxidant, antipyretic, antitumor, insecticide For asthma, diarrhea, gout, malaria, rheumatism, ulcers, vulnerary

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Ajuga laxmannii		Anticancer antimicrobial, anti-inflammatory, antioxidant, antiprotozoal, galactagogic
Ajuga lupulina	Lupulins A-D	Antimicrobial
Ajuga macrosperma	Ajugaflorins A-F, ajugamacrin E, ajugatakasin B, ajugamarin F4, ajugalides B, C, ajugalin I	Edible part (whole plant) Expectorant For nephritis, *Pyricularia oryzae* cell cyclic inhibitory
Ajuga nipponensis	Ajugamarine (=4β,18-epoxy-1α-tigloyl-6β,19-diacetoxy-12R-hydroxy-13-cleroden-15,16-olide), dihydroajugamarine	Anti-inflammatory, antitumor, expectorant, diuretic, immunity, liver protective, rheumatism
Ajuga remota	Ajugarin I, ajugarin II (**64**), ajugarin IV (**65**), ajugarin III (**66**), V, ajuganin I, clerodin	Antidiabetes, insect antifeedant, antifungal, anthelminthic, antimalarial, antimycobacterial, antioxidant, anxiolytic, cytotoxic For fever, high blood pressure, infection, malaria, pneumonia, skin diseases, stomach pain, toothache
Ajuga reptans	Ajugarin II, ajugareptasone A (**67**), ajugareptansin, ajugatansin A, ajugatansin B_1, ajugatansin D_1, ajugavensin A	Edible (leave, young shoot) Antibacterial, antitumor
Ajuga turkestanica	Ferruginol, 4β-18-nor-kaur-16-ene**,** abieta-6,8,11,13-tetraen-12-yl acetate (=ferruginyl acetate), totarol (**180**), 14,15-dihydroajugachin, 14-dihydro-15-methoxy-ajugachin B, chamaepitin (=14-dihydro-15-hydroxy-ajugachin B), ajugachin B, ajugapitin, luplin A	Antibacterial, antimicrobial, antioxidant, antiproliferative, cytotoxic For heart disease, muscle and stomach aches
Aldama discolor (=*Viguiera discolor*)	*ent*-3*α*-Hydroxy-kaur-16-en-18-ol, *ent*-7-oxo-pimara-8,15-diene-18-ol, *ent*-2*S*,4*S*-2-19-epoxy-pimara-8(3),15-diene-7*β*-ol, *ent*-7-oxo-pimara-8,15-diene-3*β*-ol	Antiprotozoal, antimicrobial
Alpinia calcarata	Zerumin A (**69**), isocoronarin D, calcaratarins A-F, (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one, laboda-8(17),11,13-trien-15,16-olide, (*E*)-labda-8(17),12-dine-15-al	Antimicrobial, anticancer, cytotoxic (KB cell) For cold

Alpinia chinensis	Labda-8(17),12-diene-15,16-dial (**29**), 14,15-epoxylada-8 (17),12-dien-16-al (**68**), coronarin E (**130**), 15-hydroxylabda-8 (17),11,13-trien-16-al, 15,16-epoxylabda-8(17), 11,13-trien-16-ol, 15-hydroxylabda-8(17),11,13-trien-16,15-olide, 14,15-dihydroxy-labda-8(17),12-dien-16-al, 12,15-dihydroxylabda-8(17),13-dien-16-al, 15-hydroxy-11,14-peroxylabda-8 (17),12-dien-16-al, dihydroxylabda-8(17),13-dien-16-al, coronarin C	Antiasthmatic, analgesic
Alpinia densespicata	Labdane glycosides: alpindenosides A-D, noralpindenosides A, B	NO production inhibitory
Alpinia formosana (pinstripe ginger)	12*E*,15,16-Bisnorlabda-8(17),11-dien-13-one, 12*E*-8(17)-labdadien-15-ol,16-al (**29**)	
Alpinia galanaga (galangal)	(*E*)-8β,17-Epoxylabda-12-ene-15,16-diol, galanolactone (**26**), galanal A (**28**), galanal B, isocoronarin D, galaganin, 8(17),12-labdadiene-15,16-dial (**29**)	Foods (flower, young shoots), for curries, soups. Beverage (herbal tea) Anticancer, antifungal, antimicrobial, cytotoxic, expectorant, α-glucosidase inhibitory, indigestion, stimulating digestion For cancers of mouth and stomach, colic, dysentery, respiratory and skin diseases
Alpinia japonica	Galanal A (**28**), B, pahangesin B	Antidiarrheal, antifungal antimicrobial, antipolyuria, antiulcer. cytotoxic, diuretic, Food preservative, NO production inhibition, tonic For aphrodiastic, hypertension
Alpinia katsumandai	Galanolactone (**26**)	Foods and beverage Antifungal
Alpinia nigra	*ent*-Labda-8(17),12-dien-15,16-dial (**29**), *ent*-8β,17-epoxylabda-12-ene-15,16-dial	Antimicrobial

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Alpinia oxyphylla	12*E*-Labda-12,14-dien-15(16)-olide-17-oic acid =	Antimicrobial, cytotoxic For abdominal disorders, anorexia, diarrhea, dementia, dieresis, α-glucose inhibitory, hypoglycemic, NO production inhibition, pain, spermatorrhea, urinary tract infection, uterine bleeding
Alpinia pahangensis	Pahangesin B	
Alpinia zermbet	Zerumin A (**69**), B, (*E*)-15,16-bisnorlabda-8(17),11-dien-13one	Beverage (herbal tea) Antimicrobial, aromatic stomachic
Ambergris	Ambroxide (**23a**) (=ambroxan, ambrox®)	Ambergris fragrance: high perfume and cosmetics for flavor Used as cosmetic flavor (skin hair, fabric flavoring spice)
Ambrosia arborescens	Diterpenoids	Cytotoxic (U937: monocyte leukemia
Amphiachyris dracunculoides (= *Gutierrezia dracunculoides*), (prairie broomweed)	18,19-Epoxy-19-hydroxy-3,13-clerodadien-15,16-olide (=amphiacrolide C), amphiacrolide A-D, gutierolide, 15,16-epoxy-7-hydroxy-8,(17),13(16), 14-labdatrien-19-oic acid (=17-hydroxyiso-lanbertianic acid)	For cold, cough
Andrographis paniculata (king of bitters)	Andrographolide A (**70**), andrographan, andrographosterin, andrographolactone, 14-deoxy-12*R*-sulfoandrographolide, neoandrographolide, 14-deoxyandrographolide, isoandrographolide, 14-deoxyandrographolide 19β-*D*-glucoside, homoandrographolide	Anticancer, angeogenic, antifertility, antimalarial, cardiovascular activity, cytotoxic (LoVor: colon cancer, NCI-H460: lung cancer), hepatorenal protective, hypoglycemic, immunological potential, liver enzyme modulatory, psychopharmacological activity, respiratory system benefit, sex hormone modulatory NF-κB inhibitory For cold, fever, hypertension, diabetes, herpes, helminth infection, peptic ulcer

Anisochilus carnosus	Coleon A, coleon Q (**71**)	Beverage (herbal tea) Anticancer, antimicrobial, antioxidant, gastric antisecretary, antiulcer, hepatotoxic, cytoprotective, stomachache
Anisochilus harmandii	4-*epi*-Triptoenzene (**72**), 12-*O*-deacetyl-6-*O*-acetyl-19-acetoxycoleon Q, pimaric acid, dehydroabietic acid (**43**), 9α,13α-*epi*-dioxyabiet-8(14)-en-18-oic acid, 7-oxo-dehydroabietic acid, 15-hydroxydehydroabietic acid, oxyabietan-18-oic acid, 12-*O*-deacetylcoleon A, 12-*O*-deacetyl-6-*O*-acetyl-18-acetyloxycoleon	Beverage (herbal tea), antimycobacterial, cytotoxic (NCl-H187 cell), tonic
Anisomeles indica	Ovatodiolide (**73**), 4-methylene-5β-hydroxyovatodiolide, 4-methylene-5β-hydro-peroxyovatodiolide, 4-methylene-5-oxo-ovatodiolide, 4α-hydroxy-5-en-ovatodiolide, 4α-hydroperoxy-5-en-ovatodiolide	Edible (tubers) Analgesic, anti-*Helicobacter pylori*, antihypertensive, anti-inflammatory, antiplatelet aggregation astringent, carminative, cytotoxic For eczema, pruritus, external afflictions, dyspepsia, fever, rheumatism, toothaches
Annona coriacea	3β,20-Epoxy-3α,16-dihydroxy-15-oxo-7-pimaren-19,6-olide	Fruit, ingredients in juices and desserts Laxative For dysentery
Annona glabra	Methyl 16β-acetoxy-19-al-*ent*-kauran-17-oate (=annoglabasin A), annoglabasin B (=16α-hyrdroxy-19-acetoxy-*ent*-kauren-17-oic acid), annoglabasin C (=16α-acetoxy-*ent*-kauran-19-oic acid-17-methyl ester), annoglabasin D (=16α-acetoxy-*ent*-kauran-19-al-17-methyl ester), annoglabasin E (=16α-hydro-19-ol-*ent*-kauran-17-oic acid, annoglabasin F (=16α-acetoxy-19-nor-*ent*-kauran-4α-ol-17-methyl ester), 16α-methoxy-*ent*-kauran-19-oic acid, 16α-hydro-*ent*-kauran-17,19-dimethyl ester	Beverage (herbal tea) Algaecide, antifungal, anti-HIV, cardiotonic infusion, insecticide sedative
Annona senegalensis	*ent*-3β-Hydroxykaur-16-ene, *ent*-kaur-16-en-19-oic acid (**47**), *ent*-16,17-deacetoxy-kauran-16-oic acid, *ent*-19-carbomethoxykauran-19-oic acid	Cytotoxic (MCF-7: breast, PC-3: prostate cancer cell)

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Annona squamosa (sugar apple, custard apple)	*ent*-17-Hydroxy-16β-kauran-19-al, *ent*-17-acetoxy-16β-kauran-19-al, 16-kauren-19-ol, *ent*-kaur-16-en-19-oic acid (**47**), 16β,17-dihydroxy-*ent*-kauran-19-oic acid (**4**) *ent*-11α-hydroxy-16-kauren-15-one (**5**), 4α-hydroxy-17,19-dinor-*ent*-kauran-16-one, 4β-hydroxy-16β*H*-18-nor-ent-kauran-17-oic acid, 4β,17-dihydroxy-16α-acetoxy-18-nor-*ent*-kaurane, annosquamosin Z, 16α*H*-*ent*-kaurane-17,18-dioic acid 17-methy ester	Fruits, ice cream, milk, beverage (herbal tea) Abortifacient, anticancer, antidiabetes, apoptosis inducing (HL-60 cell), cytotoxic (SMMC-7721, HepG2: human hepatoma cancer cells), expectorant, hypoglycemic, insecticidal, pesticidal, tonic For anemia, burning sensation, cold, cooling, diarrhea, dysentery, fainting spells, hysteria, sedative, stimulant
Annona vepretorum	*ent*-3β,19-Dihydroxy-kaur-16-ene, *ent*-3β-hydroxykaur-16-ene, *ent*-3β-acetoxykaur-16-ene, *ent*-3β-hydroxy-kaurenoic acid, kaurenoic acid	Cytotoxic (HepG2: human hepatoma; B16-F10, HL-60, non-tumor PBMC)
Aparisthmium cordatum	15,16-Epoxy-4-hydroxy-2,13(16)14-clerodatrien-17, 12-olid-18-oic acid (=cordatin), aparsthman	Antiulcer, gastroprotective
Aphanamixis polystachya	Aphanaperoxides	Cytotoxic (HepG2, MCF-7, A-549, AGS: human gastric carcinoma)
Aralia cordata	*ent*-7α-Hydroxy-8(14),15-pimaradien-19-oic acid, (**74**), *ent*-7-oxo-8(14),15-pimaradien-19-oic acid (**75**), *ent*-7β-hydroxy-8(14),15-pimaradien-19-oic acid, (**76**), *ent*-pimara-8(14),15-dien-15-oic acid, *ent*-kaur-16-en-19-oic acid, 18-nor-*ent*-pimara-8(14),15-dien-4β-ol, 18-nor-*ent*-kaur-16-en-14β-ol, *ent*-pimara-8(14),15-dien-19-ol, *ent*-pimar-15-en-8α,19-diol, 7-oxo-*ent*-pinara-8(14),15-dien-19-oic acid, 16α-hydroxy-17-isoveleroyloxy-*ent*-kauran-19-oic acid, 17-hydroxy-*ent*-kaur-15-en-19-oic acid, 15α,16α-epoxy-*ent*-kauran-19-oic acid, 16α,17-dihydroxy-*ent*-kauran-19-oic acid, 16α-methoxy-17-hydroxy-*ent*-kauran-19-oic acid, *ent*-continentalic acid, kaurenoic acid, 7-oxo-sandaracopimaric acid, pimaradienoic acid	Vegetable, salads Anti-Alzheimer, antiasthmatic, antibacterial, anti-inflammatory, antipyretic, antiulcer, COX-1, COX-2 inhibitory, Immune protective marginal acetylcholinesterase and β-site amyloid precursor protein cleaving enzyme (BACE 1) inhibitory, cytotoxic, diaphoresis, sedative For cold, sensitive constitution, lameness, lumbago, rheumatism

Araucaria angustifolia	7,13-Abietadien-18-al (=abietinal), (*E*)-communic acid (**3**)	Food (seeds), flowers, buds (medicinal use) Antidepressant, antigenotoxic, Anti-inflammatory, antioxidant, antiseptic, antiviral For anemia, fatigue, respiratory infection, rheumatism, scrofula
Araucaria araucana (pehuen)	Hibaene, kaurene, atisirene, labd-8(17)-en-15,19-diol (=imbricatadiol), 15-hydroxylabd-8(17)-en-19-al (=15-hydroxyimbricatolal), 15-hydroxylabd-8(17)-en-19-oic acid (=imbricatolic acid), 15-hydroxylabd-8(17)-en-19-oic acid methyl ester, 15-acetoxylabd-8(17)-en-19-ol, 15,19-diacetoxylabd-8(17)-ene, 15-acetoxyimbricatolal, 15-acetoxylabd-8(17)-en-19-al (=15-acetoxyimbricatolal), 15-acetoxylabd-8(17)-en-19-oic acid (=15-acetoxy-imbricatoic acid), 15-acetoxy-labd-8(17)-en-19-oic acid methyl ester, 15-methoxylabd-8(17)-en-19-oic acid methyl ester, labdanoic acid	Gastroprotective For cicatrization, contusions, ulcer, wound healing
Araucaria cookie	7,13-Abietadien-18-al (=abietinal)	Antimicrobial, antioxidant
Araucaria excelsis	7,13-Abietadien-18-ol (=abietinol)	
Araucaria heterophylla	Labda-18(17),14-diene, 13-epi-cupressic acid, 13-*O*-acetyl-13-*epi*-cupressic acid	Antiulcerogenic, cytotoxic (breast and colon cancer cell lines)
Arcangelisia flava	15,16-Epoxy-4,6-dihydroxy-2,13(16),14-clerodatrien-17,12:18,1-olide (=2-dehydroarcangelisinol), hydroxyarcangelisin, tinophyllol, 6-hydroxyarcangelisin, 2-dehydroarcangelisinol, tinophyllol, 6-hydroxyfibleucin, fibleucin, fibraurin, 6-hydroxyfibraurin, 2α,3α-epoxy-2,3,7,8α-tetrahydropenianthic acid methyl ester, 2α,3α-epoxy-2,3-dihydropenianthic acid methyl ester, fibraisin, 2α,3α-fibleucin, dihydroxy-2,3,7,8α-tetrahydropenianthic acid-2,17-lactone	Sap (sprue) antibacterial, antimicrobial, antiseptic, antitumoral, emmenagogue, expectorant, hypotensive, tonic, stimulate uterine contractions cause an abortion For indigestion, jaundice, intestinal complaints, stomach Antifungal, antimicrobial, antihypertensive cardiotonic, tonic For abortive, fatigue, hepatitis healing, malaria, worms

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Aristolochia brasiliensis	*ent*-2-Oxo-3-cleroden-15-oic acid (**77**), 17-chloro-16*R*-hydroxy-*ent*-kaurane, 2-oxo-kolavenic acid, 2-oxo-*ent*-3-cleroden-15-oic acid, kolavenic acid, *ent*-3-cleroden-15-oic acid, *ent*-clerd-3,13-dien-15-oic acid	Antioxidant, antipyretic cytotoxic (leukemia), diuretic, snake bite, tonic
Aristolochia cymbifera (Jarrinha)	Copaic acid, copalic acid, aristolochin, aristimic, arstolochic acid, aristolic acid, kolavelool, populifolic acid, 2-oxopoppulifolic acid, epi-populifolic acid, *ent*-8β-hydroxylabd-14-ene	Antimalarial, antimicrobial, antioxidant, antiprostate cancer, diuretic, sedative For menstruation
Aristolochia triangularis	*ent*-16β*H*-kaurane (=stevane A), (-)-*ent*-kaur-16-ene, (-)-*ent*-kaur-16-en-18-ol, *ent*-3β,19-dihydroxykaur-16-ene, (-)-kaur-16-en-18-al, (-)-*ent*-kaur-16-en-18-oic acid,16α,17-epoxykaurane*ent*-16β,17-epoxykaurane, *ent*-16β*H*-kauran-17-oic acid, *ent*-kauran-16α,17-diol, (-)-*ent*-kauran-16α-ol-18-al, (-)-kaur-15-en-17-ol, *ent*-15β,16β-epoxykauan-17-ol	Antifertility, antidote, antirheumatic, antiseptic, antitumor, diaphoretic, diuretic, emmenagogue For abortive, sore throats, skin diseases, snake bites, tuberculosis, wound healing
Austrocedrus chilensis	18-Hydroxyisopimar-15-ene, 18-acetoxyisopimar-15-ene, isopimara-8(9), 15-diene, isopimara-8(19), 15-dien-19-ol, sandaracopimaric acid, sandaracopimaric acid methyl ester, labda-8(17),14-dien-13-ol, 18-hydroxymanool, 18-acetoxymanool, torulosal, torulosic acid, torulosic acid methyl ester, 8(17),12,14-labdatriene, (*E*)-communic acid, (*E*)-communic acid methyl ester, (*Z*)-communic acid, (*Z*)-communic acid methyl ester, 12-oxolabda-8,13*E*-dien-19-oic acid, 12-oxolabda-8,13*E*-dien-19-oic acid methyl ester, ferruginol, dehydroabietic acid methyl ester, 7-oxo-ferruginol (= sugiol)	Antibacterial, antiprasmodial, antiproliferative, cytotoxic, diarrhea gastroprotective, sudorific

Avena strigosa	Avenaol	Food Germination stimulant
Azadirachta indica (neem)	8,11,13-abietatrien-3,7-dione (=margocin) (**78**), ninbodinin (**79**), nimosone, nimbidiol, nimbisonol, nimbione, nimbiol (**80**), nimbosone (**81**), nimbonolone (**82**), margolone, margolonone, isomargolonone, nimbosodione, 12-hydroxy-13-methoxypodocarpa-8,11,13-trien-3,7-dione (=ninbionone), 3,13-dihydroxy-13-methoxypodocarpa-8,11,13-trien-7-one (=nimbionol), 8,11,13-abietatrien-12,16-dihydroxy-3,7-dione (=margocinin) (**83**), 8,11,13-abietatrien-3β,12-dihydroxy-7-one (=margocilin) (**84**)	Antifungal, antimicrobial, to cause abortions For birth control, bloody nose, cardiovascular, diabetes, eye gum, (gingivitis), and liver problems, fever, increases immunity, intestinal worms, leprosy, loss of appetite, nature's toothbrush, skin stomach upset, skin ulcers, and blood vessels seeds: birth control, head lice, insecticide
Baccharis artemisioides	15,16-3α,4α-Diepoxycleroda-13(16),14-diene-20,12*S*-19,2β-diolide (=bartemidiolide), 15,16-epoxyclerada-3,13(16),14-triene-20,12S,19,2β-diolide (=deoxybartemidiolide)	Antirheumatism For treating traumatisms
Baccharis articulata *(= B. diptera)*	7-Oxo-16,19-dihydroxy-3,4-dehydroclerodan-15,20-diacid-dilactone, 8β-hydroxy-7-oxo-*ent*-cleroda-3-en-15,18-diacid-16,19-dilactone, 15,16-epoxy-7α,18-dihydroxy-15-methoxy-*ent*-cleroda-3-ene, *ent*-2α-hydroxy-3,13-clerodadien-15,16:18,19-diolide (=atriculin)	Antidiabetes, anthelmintic, Anti-inflammatory, antiviral, aphrodisiac, diuretic, febrifuge, gastric stimulant, tonic, digestive, hepatoprotective For male sexual impotence, female sterility, rheumatism, ulcers
Baccharis boliviensis	*ent*-3,13-Clerodadiene-15,16,17-triol (**85**), 16-hydroxybacchasalicyclic acid (**86**), 16-hydroxybaccasalicyclic acid-15-*O*-acetate (**87**), 15,16-dihydroxy-*ent*-3,13-clerodadiene-17-*O*-β-xylopyranoside (**88**), *ent*-2,4(18),13-clerodatriene-15,16-diol, *ent*-13(*Z*)-clerodene-2β,3β,4α,15,16-pentol, *ent*-3β, 4β-15,16-diepoxy-13(16), 14-clerodadien-2β-ol, *ent*-3β,4β-15,16-diepoxy-2β-methoxy-13(16),14-clerodadiene	Antibacterial, anti-inflammatory, COX-1, prostaglandin E2, (PGE2), 5-lipoxygenase (5-LOX) leukotriene C4 (LTC4), COX-2, nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) inhibitory, trypanocidal
Baccharis cassinaefolia	*ent*-15,16-Epoxy-2β-angeloyl-3β-(3-methyl-2-butenoyl)-13 (16),14-clerodadiene-18,19:20,12-diolide	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Baccharis conferta (escoba)	Bacchofertin, bacchofetone, kingidiol	Beverage (herbal tea) Antibacterial, antiobesity, diuretic, laxative, spasmolytic For cold, cramps, cold, digestive disorders, insect bite, laxative, gastrointestinal disorders, insect bites, joint pain, seizures, stomachache, toothache
Baccharis crispa	Bacrispine, 1-deoxy-bacrispine	Antifungal, antibiotic, antifeedant, antimicrobial, antioxidant, antirheumatism, antiseptic, antispasmodic For digestive, fever, hepatoprotective, liver disorders
Baccharis eggersii	17-Hydroxy-13-labden-15,16-olide	
Baccharis flabellata	2,19:15,16-Diepoxy-neoclerodan-3,13(16),14-trien-18-oic acid, 15,16-epoxy-5,10-secoclerodan-1(12)-2,4,13(16),14-pentaen-18,19-olide, 15,16-epoxy-neo-clerodan-1,3,13(16),14-tetraen-18,19-olide	Antifungal
Baccharis gaudichaudiana	Baccharіol, 15,16-epoxy-15α-methoxy-*ent*-clerod-3-en-18-oic acid, 13-*epi*-15,16-epoxy-15α-methoxy-*ent*-clerod-3-en-18-oic acid, 7-oxo-16-hydroxy-*ent*-clerod-3-en-15-oic acid methyl ester-18,19-olide	Antidiabetes, antihypertensive, anti-inflammatory, antimicrobial, antileukemia, antiseptic, hepatoprotective, hypoglycemic, diuretic, tonic For digestive
Baccharis genistelloides (=B. trimera) (escoba)	15,16-Epoxy-7α,18-dihydroxy-15-oxo-*ent*-cleroda-3-ene, 7α,15,18-trihydroxy-*ent*-cleroda-3-ene, 15,16-diacetoxy-7α,18-dihydroxy-*ent*-cleroda-3-ene, 15,16-epoxy-7α,18-dihydroxy-15-methoxy-*ent*-cleroda-3-ene, 7-hydroxy-13,14,15,16-tetranor-3-cleroden-18,19-olide, 7-oxo-3-clerodene-15,16:18,19-diolide	Beverage (herbal tea) Anti-HSV-1/VSV, antibacterial, anti-inflammatory, antifungal, antimutagenic, antithermic, antiulcerogenic, effects on bile duct, bone marrow, cardiac function, liver, pancreas, serum lipids in diabetes For analgesic, anemia, aphrodiastic, cardiovascular, diabetes, diarrhea, digestive disorders, diuretic,

		gastrointestinal, hepatic, hypertension, indigestion, inflammatory, intestinal worms, liver, kidney, renal and spleen diseases, malaria, rheumatism, sexual impotency, sore throat, stomachic, ulcers, urinary
Baccharis grisebachii	Labda-7,13*E*-dien-3β,15-diol (**6**)	Traditional medicine Antibacterial, antifungal
Baccharis hytchisonii	Bacchalineol, barticulididol	
Baccharis illinita (cha ventura)	15β-Senecioyloxy-*ent*-kaur-16-en-19-oic acid, 15β-hydroxy-*ent*-kaur-16-en-19-oic acid, 17-hydroxy-*ent*-kaur-15-en-19-oic acid	Beverage (herbal tea) Anti-inflammatory, gastroprotective For gastric disorders, infection, skin and mucosal healing, stomach ulcers
Baccharis incarum	1α-Acetoxybacchotricuneatin A, hydroxybacchotricuneatin A, *ent*-18-acetoxy-3,13-clerodadien-16,15-olide	Anti-inflammatory antioxidant, antipyretic antiseptic, wound healing For digestion
Baccharis kingii	*ent*-15,16-Epoxy-3,13(16),14-clerodatrien-18,19-diol (=kingidiol)	Juvenile hormone antagonist
Baccharis latifolia	Labda-13-en-8,15-deiol, labd-13-en-8,15-diol	Antidiabetes, anti-inflammatory, antimicrobial, antioxidant, antirheumatic, pulmonary affections, tonic, vulnerary For disinfectant, emollient, inflammation, liver disorders, rheumatism, wounds, ulcers
Baccharis lejia	Bacchalineol-18-*O*-malonate ethyl ester, patagonol-18-*O*-malonic acid, 15-hydroxy-patagonol-18-*O*-malonic acid methyl ester, 16-hydroxy-iso-patagonol-18-*O*-malonic acid methyl ester, bacchalejin 1, bacchalejin 2, bacchalejin 3, bacchalejin 4	Antifeedant, insecticidal
Baccharis leptophylla	*ent*-3β,16β,19-Trihydoxy-kaurane, *ent*-16β,17-dihydroxykauran-19-al, 16β,17-dihydroxy-*ent*-kauran-19-oic acid (**4**)	Antibacterial, antiparasitic, HIV-inhibitory, platelet aggregation inhibitory

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Baccharis linearis	Baclinal, baclinepoxide, 13-*epi*-baclinepoxide, baclinic acid, triacetyl baclinic acid	Allelopathic, antifeedant, antirheumatic, cytotoxic, insect repellent For arterial blood, catarrh, headaches, inflammations, pyorrhea, respiratory ailments, rheumatism, spasmodic, urinary vulnerary, wound healing
Baccharis macraei	15,16-Epoxy-3,13(16),14-clerodatrien-19-hydroxy-18-oic acid (=hautriwaic acid acetate), *ent*-4α-hydroxy-isobacchasmacranone, bacchasmacranone, 2β-hydroxybacchasmacranone, (-)-hardwickiic acid	
Baccharis marginalis	*ent*-2β,3β,4α-Trihydroxy-15-cledrodanoic acid (=dihydrotucumanoic acid)	
Baccharis minutiflora	*ent*-16β-Acetyl-17-nor-19-kauranol, *ent*-15-kauren-17-ol, *ent*-19-hydroxy-16β-kauran-17-al, *ent*-16α-17-epoxy-19-karanal	
Baccharis multifolia	Furanoditerpenoids	For catarrhs, urinary problems
Baccharis myrsinites	Two clerodanes	
Baccharis paniculata	*ent*-18,19-Dihydroxy-3,13-clerodadien-15,16-olide (=paniculadiol)	Antifungal
Baccharis patagonica	*ent*-5β-3,13-Clerodadien-18-oic acid 16,15-olide (=patagonic acid)	
Baccharis pedicellata	*ent*-2β,3β,4α-13,14-Dihydro-15-clerodanoic acid (=dihydrotucumanoic acid), 3,13-clerodadiene-15,16:18,19-diolide	
Baccharis petiolata	2α,3α-Dihydroxy-8-labden-15-oic acid (=2α,3α-dihydroxycativic acid	
Baccharis pingraea	Angeloyl gutierrezianolic acid, furolabda-6,8-dien-17-oic acid, furolabd-7-en-17-oic acid, 10*E*-centipedic acid, gutierrezianolic acid	

Baccharis platypoda	Platypodiol	Anti-*Plasmodium falciparum*
Baccharis rufescens	*ent*-3,19-Disuccinyloxy-kaur-16-ene	Anti-inflammatory
Baccharis salcifolia (mule fat)	15,16-Epoxy-7,13(16)14-2α,3β-dihydroxylabda-triene, *ent*-2α,3α-dihydroxy-7-labden-15-oic acid (**89**), *ent*-15-hydroxy-3,13*E*-cleroda-dien-17-oic acid (=bacchasalicylic acid), *ent*-5-hydroxy-3,13*E*-clerodadien-17-oic acid 15-*O*-β-xylopyranoside, salicifolic acid, 5-hydroxy-6-hydrosalicifolic acid	Food (young shoot), Beverage (herbal tea) Anti-inflammatory, antirheumatic, antisyphilitic, diuretic, female germination inhibition, hygienic agent For bruise, colic, diarrhea, diaphoretic, dysentery, eye fevers, headache, inflammation, insect stings, wound healing
Baccharis salzmannii	6β,15,18,19-Tetrahydroxy-*ent*-cleroda-3-ene, 6β,15,18-trihydroxy-*ent*-cleroda-3-ene, *ent*-kaur-16-en-19-oic acid (**47**)	
Baccharis sarothroides	15,16-Epoxy-2,19-dihydroxy-3,13(16),14-clerodatrien-18-oic acid (=*ent*-2α-hydroxy-hautriwaic acid)	Seed: beverage (herbal tea) Cytotoxic (human carcinoma inhibitory), muscle relaxant For cold, refreshment
Baccharis scoparia	8β,13*R*-Epoxy-15-labdanal, *ent*-12α*H*,20:15, 16-diepoxy-7-oxo-3,13(16),14-clerodatrien-18,19-olide (=12-*epi*-conycephalide), baccharascoparone	
Baccharis sternbergiana	*ent*-8,17-Labdene-2α,15-diol, *ent*-15-hydroxy-8,17-labdene-2-one	
Baccharis thymifolia	6,10-(*E,E*)-Thymifodioic acid	
Baccharis tola	Erythroxylol-A, *ent*-bayer-15-en-18-ol, erythroxylol-A 14,15-oxide, 19-hydroxy-13-epi-manoyl oxide, *ent*-bayer-15-en-17,19-diol, *ent*-4α-hydroxy-19-norbeyer-15-ene, *ent*-beyer-15-en-18,19-diol, 15β,16β-epoxy-*ent*-beyeran-18-ol	Antibacterial, anti-inflammatory, protein synthesis
Baccharis tricuneata	Bacchotricuneatin A-D, *ent*-15,16-epoxy-3,13(16),14-clerodatrien-18-ol (=bacchalineol)	Antidiabetes, antianernic, antirheumatic, antiseptic, disinfectant For bronchitis, diabetes, headache, skin infections, wound healing

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Baccharis tucumanensis	*ent*-2β,3β,4α-Trihydroxy-13*Z*-cleroden-15-oic acid (=tucumanoic acid)	Abortive agent, anti-inflammatory
Ballota inaquidens	Hipanolone, ballonigrine (**90**)	Antibacterial, antifungal antispasmodic, antiulcer, sedative
Ballota saxatilis subsp. *saxatilis*	Hispanolone, dehydrohispanolone, ballonigrine	Antibacterial, antifungal
Barbacenia flava	Barbacenic acid, 3-deoxybarbacenic acid, 3-oxo-17-carboxy-3,18-secobarbacenic acid	
Beyria leshenaultii	*ent*-Kaurene-3β,19-diol	For tuberculosis
Bideus pilosa	Phytyl heptanoate	Beverage (herbal tea) Emmenagogue For abdominal troubles, dysentery, rheumatism, sore eyes, swollen, toothache, ulcers
Bifucaria bifurcate (brown algae)	Bifurcadiol (**91**), 12-hydroxygeranylgeraniol	Cytotoxic (A-549, HCT, SK-L-2, SK-OV-3, XF-498: human tumor cell lines)
Bowdichia nitida	6α,7α-Diacetoxyvouacapane	Anti-*Plasmodium falciparum*
Bromelia pinguin	7β,8β-Dihydroxypimar-15-en-3-one (**92**), 3-oxophyllocladan-16ξ-ol, phyllocladan-16α,19-diol	Fruit, edible (fresh shoot: cooked or raw), vinegar, beverage (refreshing drink) Antibacterial, cytotoxic
Bux sempervirens	Phytol	Decoction: antirheumatism Infusion: respiratory, sedative
Caesalpina echinata	Echinalides H-U	NK-κB inhibitory
Caesalpinia bonduc	Casalpinolide C-E, cassane furanoditerpene	Cytotoxic (MCF-7: DU145: prostate carcinoma, C33A: cervical carcinoma, Vero: fibroblast cells)
Caesalpinia decapetala	Caesaldecapes A, B	Cytotoxic (KB cell)
Caesalpinia echinata	Cassanes, echinalides H-U	Anti-*Leishmanial amazonensis*, NK-κB inhibitory
Caesalpinia furfuracea	Caesalfurfuric acid A	Antimicrobial

Caesalpinia minax (seed: kushilian)	Caesalmine A (**93**), B	Anti-*Plasmodium falciparum*
	Sucuriniranes (G-I)	Cytotoxic (Hela, HepG-2 cancer cell lines)
	Caesalmins C-G, caesalmanaxin A-L spirocaesalin, macrocaesalmin, neocaesalpins I-N, neocaesalpins S-U, neocaesalpins AA-AE, bonducellpin D, methyl 1α,7β-diacetoxy-5α,12α-dihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate, methyl 7β-acetoxy-1α,5α,12α-trihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate, 12α-ethoxy-1α,6α,7β-triacetoxy-5α,14β-dihydroxycass-13(15)-en-16,12-olide	Cytotoxic (Du145, K562 cancer cell lines) For cold, dysentery, fever
Caesalpinia pulcherrima	Pulcherrimins A-D, isovoiacapenol, 6β-cinnamoyl-7β-hydroxyvouacapen-5β-ol, pulcherrimin E-F, neocaesalpin E-G, 6β-hydroxy-isovouacapenol, 7β-acetoxy-6β-hydroxy-iso-vouacapenol, iso-vouacapenol monoacetate	Antibacterial, antifungal For liver disorders, mouth and throat ulcers
Caesalpinia sappan	Caesalsappanins O-S, morcaesalpinin, phanquinin A (**94**), B-K, phangininoxys B, C, 11-oxophanginin, phanginin U	Antibacterial, anthelmintic, anti-inflammatory, antimalarial, antineoplastic, antioxidant, antiplasmodial, antiproliferative, blood stagnation, complementary, cytotoxic (HCT: colon; MCF-7: breast cancer cell lines), dysmenorrheal, hepatoprotective, hypoglycemic, immunomodulatory, xanthine oxidase-inhibitory For diarrhea, dysentery, tuberculosis
	Cassanes, furanoditerpenoids, tomocinon, tomocinol	Antimalarial
Caesalpinia sylvestris	Casearin B	Cytotoxic (HePG2)
Calceolaria ascendens (topa-topa)	Dehydroabietinol, 19-malonyloxydehydroabietinol, 4-*epi*-dehydroabietic acid	Antibacterial, stomachache, sweeteners, tonic,
Calceolaria foliosa	*ent*-8,15-Isopimaradien-18-ol	
Calceolaria latifolia	*ent*-Isopimara-8(9),15-dien-18-ol,*ent*-isopimara-8(9),15-dien-19-ol, *ent*-stemara-13(14)-en-19-ol, *ent*-stemara-13(14)-en-18-ol	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Calceolaria lepida	19-Pentyloxy-*ent*-pimar-15-en-9α-ol, lapidate, *ent*-pimara-9 (11), 15-dien-14-ol, *ent*-pimar-15-en-9α,19-diol, *ent*-stemar-13(14)-en-19-ol, methyl *ent*-stemar-13(14)-en-19-oate, methyl 19-malonyloxy-*ent*-pimar-15-en-9α-ol	
Calceolaria pinifolia	19-Malonyloxy-*ent*-iso-pimara-8(9),15-diene, methyl 19-manoyloxy-*ent*-iso-pimara-8(9),15-diene, 4-*epi*-dehydroabietinol, *ent*-iso-pimara-9(11),15-dien-19-ol, dehydroabietic acid (**43**), dehydoabietinol, malonyloxy-8,11,13-abietatriene, 8,15-iso-pimaradien-19-ol, methyl 19-malonyloxy-*ent*-iso-pimara-9(11),15-diene,19-malonyloxy-*ent*-iso-pimara-9(11),15-diene	Antimicrobial, antiMRSA, astringent, bactericidal, stomachic, sweeteners
Calceolaria talcana	Diterpene mixtures	Insecticide
Callicarpa candicans (fish killing dynamite)	Callicarpone	Antiasthma, antimicrobial, fish-killing, insecticidal For abdominal troubles, amenorrhea, depuration, fever, emmenagogue, skin diseases
Callicarpa macrophylla	Macrophypene A (**95**), B-E, calliphyllin, 14α,18-dihydroxy-7,15-iso-pimaradiene, 8α,9α,13α,14α-diepoxyabietan-18-oic acid, 7α-hydroxydehydroabietic acid, 7-oxodehydroabietic acid, abieta-8,11,13,15-tetraen-18-oic acid, 17-acetoxy-16β-hydroxykaur-3-one, *ent*-16α,17-dihydroxy-kauran-3-one, 3-oxoanticopalic acid	Fruit (sweet and succulent) Nervous growth factor stimulant For diarrhea, dysentery, fever, gastrointestinal bleeding, indigestion, rheumatism, stomachic
Callitris columellaris	Sandaracopimaric acid (**35**), (*Z*)-communic acid, (*E*)-communic acid (**3**), isopimaric acid, 7-oxo-4-epidehydroabietic acid, labda-8(17),13(16), 14-trien-19-oic acid	Cosmetic, perfumery Antianxiety For aromatherapy, cold, cramps, cuts, sores, stomachic
Callophycus serratus (red algae)	Bromophycolides A-U, debromophycolide A, callophycoic acids A-H, callophycols A, B	Antibacterial, anticancer, antifungal antimalarial, antitubecular

Calocedrus formosana	(*E*)-Communic acid (**3**) isocuressic acid, 15-acetoxycommunic acid, agathoral, pinosolide acid, 13S-labda-8(17),14-dien-13*Z*-*p*-hydroxy cumanoxy-19-ol, 16-hydroxylabda-8(17),13-diene-15,19-dioic acid butenolide, sugiol	Anti-inflammatory, antitumor
Calocedrus macrolepis	Calomacrins A-C, labdanes, 15-methoxy-pinusolidic acid, lambertianic acid, agatholol, 15,16-dihydroxy-8(17), 13*E*-labdadien-19-oic acid	Antifungal, cytotoxic (A-549, HL-60, MCF-7, SMMC-7721, SWe80 cancer cell lines)
Canabalia gladiata	Gibberellin A_{21}, gibberellin A_{59}, canavalisoide, gladiatosides A_1-A_3, B_1-B_3 C_1, C_2	Vegetables, beverage (herbal tea) Anti-inflammatory For abdominal dropsy, ache, asthma, atopic coughs, dermatitis, dysentery, epilepsy, headache, inflammatory diseases, intercostal neuralgia, kidney disease, kidney-related lumbago, obesity, schizophrenia, stomachache, swelling vomiting
Canistrocarpus cervicornis	4*R*,9*S*,14*S*-4α-Acetoxy-9β,14α-dihydroxydolast-1(15),7-diene (**96**)	Antitoxin (snake venom)
Caryopteris divaricata	Clerodin-I, caryoptin, dihydrocaryoptin, cledorin hemiacetal, caryoptin hemiacetal, caryopinol	Antifeedant
Caryopteris terniflora	*ent*-7β,11α,14-Trihydroxy-11β,20-epoxy-kaur-16-en-15-one-18-al, *ent*-7β, 14-dihydroxy-11α-methoxy-11β-20-epoxy-kaur-16-en-15-one-16-al	Antifungal, antitumor For cold, headache, rheumatic pains, scrofula
Casearia corympsa	18,19-Diacetoxy-3,13(16), 14-clerodatrien-2-one	
Casearia graveolens	Graveospene A -I	Cytotoxic (HepG2)
Casearia grewiifolia	Caseargrewiins A-D, *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*, 18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6-methoxy-2-(2-methylbutanoyloxy)-cleroda-3,13(16),14-triene, *rel*-(2*S*,5*R*,6R,8S,9S,10R, 18S,19R)-18,19-diacetoxy-18,19-epoxy-6-hydroxy2-(2-methylbutanoyloxycloreda-3,13 (16),14-triene	Antifungal, antimicrobial antitumor, cytotoxic (KB, BC1, NCI-H187), febrifuge, tonic
Casearia pitumba	18,19-Epoxy-3,13(16),14-clerodatriene-2,6-hydroxy-18,19-diacetate (=pitunbin)	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Casearia sylvestris	Casearins B, G, U, V, *ent*-kaurane glucosides, sylvestrisides A, B, (−)-hardwickic acid, (−)-patagonic acid, *ent*-kaur-16-ene-3*β*,18-diol, 15-hydroxy-3-cleroden-2-one	Analgesic, anticlastogenic activities, anti-inflammatory, anti-snake venom, antitumoral, antiophidian, antiulcer, DNA-modifying activities For appetite suppressant, diuretic, weight loss product, snake bite, **t**rypanocidal
	Casearins B	Reaction oxygen species (ROS) production inhibition
	Oxygenated tricyclic clerodanes	Cytotoxic (Sarcoma 180, B-16/F-10)
Casimiella ampla	Humirianthol	
Casimiella rupestris	Humirianthenolide A-F, casearupestrins A−D, 2,7-di-*O*-acetyl-casearupestrin A, 2,6-di-*O*-acetyl-casearupestrin D, doxorubicin	Cytotoxic (MDA/MB-435: melanoma; SF-295 glioblastoma cancer cell lines)
Caulerpa brownie (green algae)	Cyclic, acyclic diterpenoids	
Caulerpa lentillifera (green algae: Umibudo)	Phytol, phytadienes, unidentified terpenoids	Foods, salads Antimicrobial, antioxidant
Cedrus atlantica	Sandaracopimaric acid, abietic acid (**2**), iso-pimaric acid, levopimaric acid, palustric acid, dehydroabietic acid (**43**), neoabietic acid, 18-norabieta-8,11,13-trien-4-ol, 7-oxodehydroabietinol, 8,11,13-abietatriene-15,18-diol, 7α-hydroxydehydroabietinol, 8,11,13-abietatriene-7α-ol	Flavoring agent Antibacterial
Cedrus deodora	8,11,13-Abietatriene-7α, 18-diol, 7β,15-dihydroxy-8,11,13-abietatrien-18-oic acid	
Celastrus hypoleucus	7-Deoxynimbidiol	Cytotoxic (A549, CNE, MCF, HCT116, HeLa)
Ceriops tagal	Tagakenes A-F	Cytotoxic (a panel of tumor cell lines)
Chamaecyparis formosensis	Formosanoxide, formosanolide, formosanone, formosanic acid, 12-hydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial (**97**)	Compound flavor Antifungal, antimicrobial, larvicidal (mosquito), NO production inhibitory
Chamaecyparis nootlatensis	8-epi-Manoyl oxide, 8,13-diepimanoyl oxide	

Chamaecyparis obtusa	(*E*)-communol, ferruginol, hamaecydin, 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial, hinokione, 13-*epi*-trulosal, 13-*epi*-cupressic acid, 13-*epi*-truolsol, hinokiol, 3-*epi*-triptobenzene, 1α,2α-epoxyhinokione (**98**), camaecydin (**99**)	Compound flavor, soap flavor, bath additives Anticancer, antifeedant, antifungal, cytotoxic (BGC-323, Hela, A549 cancer cell lines), bactericidal, insecticide
Chamaecyparis pisifera	8,11,13-Abietatriene-12,15-diol (=15-hydroxyferruginol), 20-nor-1(10),2,8,11,13-abietapentaen-12-ol, 8,11,13-abietatriene-12,20-diol, pisiferol, pisiferal, pisiferic acid, *O*-methoxypisiferic acid, pisiferanol, pisiferadiol (=20*S*-hydroxypisiferanol), 12-deoxypisiferanol, pisiferin, iso-pisiferin, 1β-hydroxyisopisiferin, 12-hydroxy-20-*nor*-abieta-1(10),2,8,11,13-pentaene, (10*S*,12-dihydroxy-9(10 → 20)-*abeo*-abieta-8,11,13-triene), 1β-hydroxy-iso-pisiferin (=1*R*,12-dihydroxy-9(10 → 20)*abeo*-abieta-8,10(20),11,13-tetraene), dimethylamine salt of *O*-methylpisiferic acid, rel-(8*R*,10*R*,20*S*)-8,10,20-trihydroxy-9(10→20)-abeo-abieta-9,3-dien-12-one, 8(14),15-pimaradien-19-oic acid	Antibacterial, antimicrobial
Chasmanthera dependens	1β,4α,5α,12α*H*,15,16-Epoxy-4,8-dihydroxy-2,13(16)-clerodatriene-17, 12:18,1-diolide (=8β-hydroxycolumbin)	Androgenic anticoagulant, antimicrobial, antioxidant, fertility enhancing, larvicidal, thrombolytic
Chiliotrichium rosmarinifolium	*ent*-15,16-Epoxy-3,4-seco-4,13(16)14-halimatrien-3-oic acid (=secochilotrin), 8-hydroxy-7-labdan-15-oic acid, geranyl geraniol derivatives, 3-iso-labdanes, 3-seco-iso-labdandes, *Z*-clerodanes	
Chloranthus henryi	Henrilabdane C	Cytotoxic (BEL-7402: hepatoma; BGC-823: gastric carcinoma; HCT-8: colon cancer cell lines)
Chrisothamnus nauseosus	9,13-Epoxy-18-hydroxy-7-labden-15-oic acid (=18-hydroxygrindelic acid), 9,13-epoxy-18-oxo-7-labden-15-oic acid (= oxogrindelic acid), 9,13-epoxy-18-(methylpropanoyl)-7-labden-15-oic acid, 9,13-epoxy-18-succinoyl-7-labden-15-oic acid, 3α-hydroxy-7,13*E*-labdadien-15-oic acid, sempevirenic acid, 3β-hydroxy-7,13*E*-labdadien-15-oic acid, 3α-hydroxy-7,13*E*-labdadien-15-oic acid, 3-oxo-7,13*E*-labdadien-15-oic acid	Chewing gum For chest pains, colds, coughs, diarrhea, fevers, internal injuries, menstrual cramps, stomachache, toothache

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Chromodoris kuniei	Spogiane diterpenoids	Cytotoxic (NBT-T2)
Chromolaema laevigata	*ent*-6β,7α-Dihydroxy-3,13*Z*-clerodadien-15-oic acid (=6β,7α-dihydroxykolavenic acid), *ent*-6β-angeloyl-7α-acetoxy-3,13*Z*-clerodadien-15-oic acid	Analgesic, antifungal, anti-inflammatory, purgative For influenza, intestinal disorders, itching, menstrual regulator, stomatitis, swelling, toothache, thrush, pruritus, ulcerative
Cinnamomum cassia	Cinncassiol A (**100**), cinncassiol-A-19-*O*-β-*D*-glucoside (**101**), anhydrocinnazeylanol, anhydrocinnazelanine, cinnacassiol C (**102**), cinncassiol C-19-*O*-β-*D*-glucoside, cinncassiol C_2 , cinncassiol C_3, cinncassiol D_4 **(103)**, cinncassiol D_4-19-*O*-β-*D*-glucoside (**104**), cinncassiol D_1, cinncassiol D_1-19-*O*-β-*D*-glucoside, 4β-hydroxycinncassiol cinncassiol D_2, 4β-hydroxycinncassiol D_2-19-*O*-β-*D*-glucoside, cinncassiol D_3, cinncassiol E	Food flavor, beverages (herbal tea), cookies, bread, chewing gum, crude drug Analgesic, antibiotic, antiinfectious, anti-inflammatory, antineuralgic, antiseptic, antispasmodic, antiviral, aphrodisiac, bactericidal, cholagogues, cicatrisant, decongestant, expectorant, digestive, emmenagogue, febrifuge, hepatic, hypotensive, insecticide, muscle relaxant, nervine, sedative, stimulant, stomachic, sudorific, tonic, vermifuge
Cinnamomum zeylanicum	Cinnzeylanol, cinnzeylanine, cinncassiol B, cinnzeylanol-19-*O*-β-*D*-glucoside	Beverages, food flavor, and flavor and aroma enhancer for beverage, cookies, bread, chewing gum, source, spice Analgesic, anti-Alzheimer's disease, antiaging, antibiotic, antidiabetic, antifungal, antioxidant, antiparasitic, antiseptic, antispasmodic, aphrodisiac, astringent, bactericide, cardiac, carminative, emmenagogue, insecticide, nephropathy, stimulant, stimulating appetite, stomachic, tonic, vermifuge For cold, diarrhea, headaches, respiratory complaint, muscular aches and pain, stomach upset

Cistus albidus	13-*epi*-Manoyl oxide	Antimicrobial
Cistus creticus	Manoyl oxide, 13-*epi*-manoyl oxide, 8,13-epoxy-15,16-dinorlabda-12-ene, 3β-hydroxy-13-*epi*-manoyl oxide, labd-7,13-dien-15-ol, labd-7,13-dien-15-yl acetate, 3β-acetyl-13-*epi*-manoyloxide, labd-13-en-8α,15-diol, labd-13-en-8α-ol-15-yl acetate	Anthelmintic, antibacterial, antifungal, antioxidant, cytotoxic (cervix, breast, melanoma cancer cell lines)
Cistus incanas (Ladano)	(5,8,9,10*R*)-Labd-13*E*-ene-8α,15-diol, (5,8,9,10*R*)-labd-7,13*E*-dien-15-ol, (5,8,9,10*R*)-labda-7,13*E*-15-yl acetate, 8,13-epoxylabd-14-ene, 13-*epi*-8,13-epoxylabd-14-ene, labd-14-ene-8,13-diol, 13-*epi*-sclareol	Beverage (herbal tea) Antibacterial, antifungal, antiviral, antioxidant, antiviral, cytotoxic (KB, P-388, NSCLC-N6 cancer cell lines), myorelaxant
Cistus labdaniferus	Dehydroabietic acid (**43**), 8-abieten-18-oic acid (**105**)	Antimicrobial, anti-leishmanial, antimultidrug resistant For malarial symptoms, fatigue, muscular aches
Cistus ladanifer	6-Acetoxy-7-oxo-8-labden-15-oic acid, 7-oxo-8-labden-15-oic acid, oxocativic acid	Antibacterial, antifungal, antioxidant, antiviral, analgesic effects, spasmolytic
Cistus laurifolius	6β,8-Dihydroxy-*ent*-13*E*-labden-15-oic acid (=laurifolic acid), salmantidiol, salmantic acid, methyl salmantiate	Antioxidant
Cistus monspeliensis	Cistodiol, cistodioic acid	Antiaging, antibacterial, anticancer, antioxidant, fatigue, isogenic, malarial symptoms, muscular aches, myorelaxant, spasmolytic
Cistus mospeliensis	13-*epi*-Manoyl oxide, manoyl oxide	Cytotoxic
Cistus palhinhae	*ent*-5β,10β,13ξ-2-keto-3-cleroden-15-oic acid	For dementia, dental decay, elephantiasis, epilepsy, fatigue, filariasis, gastrointestinal, malarial symptoms, muscular aches, parasitic infections, respiratory disorders
Cistus parviflorus	Manoyl oxide, 13-*epi*-manoyl oxide	Antioxidant
Cistus populifolius	*iso*-Dehydropopulifolic acid (**106**), *epi*-hydroxy-populifolic acid (**107**), β-methoxypopulifolic acid (**108**), hydroxypopulifolic acid (**109**), α-methoxypopulifolic acid (**110**)	Analgesic, antibacterial, antioxidant, antiviral, spasmolytic

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Cistus salviifolius	Manoyl oxide, 13-*epi*-manoyl oxide, *Z*-ferruginol, iso-dehydro-populifolic acid, epi-hydroxypopulifolic acid, β-methoxypopulifolic acid, hydroxypopulifolic acid, α-methoxypopulifolic acid	Antibacterial, anticancer, antifungal, antioxidant For colds, diarrhea, digestive, fevers, inflammatory, rheumatism, skin diseases
Clerodendron brachyathum	Clerodinin A, B, D, clerodin, clerodinion C, clerodiol	Antihypertensive, diuretic
Clerodendron calamitosum	*ent*-4β,18:11*R*,16:15,16*R*-Triepoxy-14-clerodene-3α,6β,19-triol (=3-epicaryoptin), caryopinol, caryoptin, *ent*-4β,18:11*R*,16:15,16*R*-triepoxy-14-clerodene-3α,6β,19-triacetate	Antifeedant
Clerodendron inerme	*ent*-3,13-Clerodadien-16,15-olide-18-oic acid (=clerodermic acid, inermes A, inermes B, 14,15-dihydro-15β-methoxy-3-epicaryoptin, 14,15-Dihydro-15-hydroxy-3-*epi*-caryoptin	Antiplasmodial, antifeedant (mosquito)
Clerodendron infortunatum	4β,18:11*R*,16*R*;15,16*R*-Triepoxy-14-clerodene-6β,19-diacetate (=clerodin)	Anticonvulsant, antidandruff, antidiabetic, antifeedant, antihyperglycemic, antipyretic, ascaricide, insecticidal, laxative, colic, tumors, vermifuge For diarrhea, gravel, headache, liver disorders, malaria, scabies, scorpion, sting and snake bite, skin diseases sores, spasm, tumors
Clerodendron trichotomum	Clerodendrin A (= (2*R*,3*S*,4*R*,5*R*,6*S*,9*R*,10*R*, 11*S*,13*S*,16*R*)-6,19-diacetoxy-3-[(2*R*)-2-acetoxy-2-methylbutyryl-oxy]-4,18:11,16:15,16-triepoxy-7-clerodene-2-ol (=clerodendrin A) (**111**), 15,16-dehydro-teuvincenone G, trichotomin A, 2α-hydrocaryopincaolide F, villosin C, 15-dehydro-17-hydroxycyrtophyllone A, demethylcryptojaponol, 6β-hydroxydemethylcryptojaponol	Foods (leaves) Analgesic, antihypertensive, anti-inflammatory, antioxidant, antirheumatic, NO production inhibitory, sedative
	Trichotomone	Cytotoxic (A549, GC-832, Jurkat, 293T WT)

Clerodendrum bungei	Abietane diterpenoids, uncinatone	Insect antifeedants, cytotoxic (B16: murine melanoma, HEK-293: human epithelial kidney; HGC-27: human gastric cancer cell lines) For beriberi, boils, cuts, eczema, hemorrhoids, hypertension, pesticidal, postpartum hemorrhage, prolapse of the uterus, rheumatism, wounds healing
Clerodendrum kiangsiense	Abeoabietan	Cytotoxic (A-549, HL-50, MCF-7, SMMC-7721)
Cloranthus serratus	Serralabdanes	Antioxidant, NO production inhibitory
Cluytia richardiana	Cluytene C-E, saudin	Antidiabetes, hypoglycemic
Cneorum tricoccon (=Spurge olive)	Cneorubin U, W1, X, Y	Purgative, rubefacient
Coffee seeds	Cafestol (**7**)	Phosphorylation and NO production inhibitory
	Kahweol (**9**)	Cytotoxic (HepG2) Antinociceptive COX-II, inducible NO synthase (iNOS) inhibitory, peripheral
	Cafestol palmitate	
Coffea arabica	Cafestol (**7**), 16-*O*-methylcafestol (**8**), kahweol (**9**), 16-*O*-methylkahweol, cafemines A-G, cafemides A-G, mazambioside (**112**), cafestol palmitate	Beverage (coffee) Antiangiogenic, anticancer, anti-inflammatory, antinociceptive, antiosteoporosis, antitumorigenic, chemoprevention, cytotoxic (HePG2), α-glucosidase inhibitory, hypercholesterolemia, COX-2 and NO synthase (iNOS) inhibitory, NO production, and phosphorylation inhibitory
Coffea canephora (= *Coffea robusta*)	Cafestol (**7**), kahweol (**9**), 16-*O*-methylcafestol (**8**), cafestol palmitate	Food, beverage (coffee) Analgesic, anorexic, antidotal, aphrodisiac, cardiotonic, CNS-stimulant, counterirritant, COX-II and NO synthase (iNOS) inhibitory, cytotoxic (HePG2), diuretic, galactagogic, α-glucosidase inhibitory, hypercholesterolemia, hypnotic, nervine immune protective, NO production and phosphorylation inhibitory, uretic effects, vomiting

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
		controls For asthma, atropine poisoning, fever, flu, headache, immune protective, jaundice, malaria, migraine narcosis, nephrosis, opium poisoning, sores, vertigo
Coffea congensis	Cafestol (**7**), kahweol (**9**)	Beverage (coffee) Anticarcinogenic, serum cholesterol raising
Coffea liberica var. *dewevrei*	Cafestol (**7**), kahweol (**9**), 16-*O*-methylcafestol (**8**)	Beverage (coffee) Anticarcinogenic For serum cholesterol raising
Coffea liverica var. *liberica*	Cafestol (**7**), kahweol (**9**)	Beverage (coffee) Anticarcinogenic For headaches, serum cholesterol raising, sore eye
Coffea pseudozamguebariae	Cafestol (**7**), kahweol (**9**), mazambioside (**112**)	Beverage (coffee), bitterness Anticarcinogenic For headaches, serum cholesterol raising
Coffea racemsa	Cafestol (**7**), kahweol (**9**),	Beverage (coffee) Anticarcinogenic For headaches, serum cholesterol raising
Coffea salvatrix	Cafestol (**7**), kahweol (**9**),	Beverage (coffee) Anticarcinogenic For headaches, serum cholesterol raising
Coffea sessilifora	Cafestol (**7**), kahweol (**9**),	Beverage (coffee) Anticarcinogenic For headaches, serum cholesterol raising
Coffea stenophylla	Cafestol (**7**), kahweol (**9**), 16-*O*-methylcafestol (**8**), 16-*O*-methylkahweol	Beverage (coffee), Anticarcinogenic For headaches, serum cholesterol raising

Coffea vianneyi	Mascarol (**113**), mascaroside (**114**)	Beverage (coffee)
Coleus aquatic	6,11,12,14,16-Tetrahydroxy-5,8,11,13-abietatetraen-7-one (=coleon C), 11,12,14,16-tetrahydroxy-8,11,13-abietatriene-6,7-dione (=coleon D), 6,11,12,14-pentahydroxy-5,8,11,13-abietatetraen-7-one (=coleon U)	
Coleus argentatus (=*Plectranthus argentatus*)	6β-Formoxy-7,12-trihydroxy-8,12-abieta-diene-11,14-dione (=6β-formoxy-7α-hydroxyroyleanone, 6β,7α,12-trihydroxy-8,12-abietatriene-11,14-dione (=6β,7α-dihydroxyroyleanone), 7α-acetoxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione (=7α-acetoxy-6β-hydroxyroyleanone), 7α-fromyloxy-6β-hydroxyroyleanone, 8α,9α-epoxy-6,12-dihydroxy-5,12-abietadiene-7,11,14-triene (=8α,9α-epoxycoleon U quinone)	Antimicrobial For sensor
Coleus aromaticus (*Karpurvalli*)	Phytol	Functional foods, flavoring for stuffing of meat and poultry beef and lambs Antimicrobial, antioxidant
Coleus carnosus (=*Plectranthus amboinicus*)	6β,20-Epoxy-6,11,12-trihydroxy-8,11,13-abietatrien-7-one (=carnosolone), 3β,6,11,12,14-pentahydroxy-5,8,11,13-abietatetraen-7-one (=coleon S), coleon U, royleanone, 6,7-dehydroroyleanone, 7α-acetyloxyroyleanone, horminone, 6β-hydroxyroyleanone, 7α-acetyloxy-6β-hydroxy-royleanone, 6β,7α-dihydroxyroyleanone, 7α-acetyloxy-6β,20-dihydroxyroyleanone, coleon V, 6α-carnosolone, 12-trihydroxy-6,20-epoxymethanoabieta-8, 11, 13-trien-7-one	Food (raw or cooked, spice, poterb) Anti-inflammatory, antimicrobial, antiseptic, antitumor, antiviral, antituberculosis, bronchodilator, insect repellent For asthma, bronchitis, burns, cholera, colds, congestive, convulsions, cough, digestive problem, epilepsy, fevers, headaches, heart failure, meningitis, menstrual pain, skin allergy, sore, ulcers, urinary diseases, wound healing
Coleus coerulescens	Coleon O, 12-deacetoxycoleon R, 17(15→16)abeo-3α,18-diacetoxy-6β,7α,16-trihydroxyroyleanone, 17(15→16),9(4→3)-bisabeo-6,7,16-trihydroxy-royleanone, 3α,18-deacetoxy-6β,7α,16-trihydroxyroyleanone	
Coleus forskohlii	Forskolin (**10**), coleonone, 8α,13*R*-epoxy-14-labden-15-one, 8,13-epoxy-9α-hydroxy-14-labden-15-one (=coleol), coleonol F, coleonols B, D, F, coleonol E (**115**), 8,13-epxy-6β-hydroxy-7β-acetoxy-14-labden-11-one, 8,13-epoxy-6β-hydroxy-14-	*Herbal medicines Adenylyl cyclase effect, antiasthma, antibacterial, antidepressant, antidiabetic, antidrepanocytary, antihistamine, antihypertensive, anti-inflammatory, antimetastatic, antiproliferative,*

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
	labden-11-one, coleosol, folskolins G-J, 13-episclarenol, folskoditerpenosides A, B, 14-deoxycoleon U, demethylcryptojaponol	*antioxidant, breast milk-stimulant, bronchodilation, cancer prevention, cardiovascular ailments, cardiovascular cloning and molecular genetics, cytochrome P450 and nuclear receptor effect, fat burning supplement, hepatoprotective effect, insulin and thyroid hormone secretion increasing, NO scavenging, platelet aggregation, relaxative, renal, thyroid effects, vaculogenic properties* *For angina, eczema, hypothyroidism, heart health, psoriasis, weight loss*
Coleus fredericii	Fredericones A-D	
Coleus igniarius	Coleons A, B	For inflamed eyes, pain, skin condition
Coleus kilimandschari	Coleon F	Antifungal, antimicrobial
Coleus somaliensis (=*Plectranthus somaliensis*)	3β-Acetoxy-6,11,12,14, 16-tetrahydroxy-5,8,11,13-abietatetraen-7-one (=coleon H), 3β-acetoxy-11,12,14,16-tetrahydroxy-8,11,13-abietatriene-6,8-dione (= coleon I), coleon J, 3β,17-diacetoxy,11,12,14,16-tetrahydroxy-8,11,13-abietatriene-6,8-dione (=coleon K), coleon O, 19-acetoxycoleon Q	
Coleus xanthanthus	Coleon U-11-acetate, 16-acetoxycoleon U-11-acetate, xanthathusins F-K, coleon U, 16-O-acetylcoleon C, coleon U quinone, 8α,9α-epoxycoleon U-quinone, xanthanthusin E	Cytotoxic (K562 human leukemia cell line) For arthritis, colds, coughs, injuries, neurasthenia, rheumatism, scabies, snake bites, tuberculosis
Coleus zeylanicus	6β,7β-Dihydroxy-royleanone,7β-acetoxy-6β-hydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone	For diarrhea
Conyza aegyptia	16,15-Epoxy-3β,4α,7β, 10β-tetrahydroxy-12β*H*,13(16),14-clerodadien-20,12-olide	
Conyza blini	14,15-Dinor-labdan-13-one-8-*O*-*α*-L-arabinopyranosyl-2,8,19-trihydroxy-3,13-cleroda-dien-15,16-olide (=blinin)	For chronic bronchitis and other inflammatory diseases, gastroenteritis

Conyza pyrifolia (=*Microgrossa pyrifolia*)	1-Acetyl-6*E*-geranygeraniol-19-oic acid, 6*E*-geranylgeranyol-19-oic acid , (*E*)-phytol, 3α,4α:15,16-dieoxy-10-oxo-5,10-seco-13(16),14-clerodadien-20,12-olide (=10α-angelyoyl-10-desoxopyrrhopappolide	Antimalarial, anti-inflammatory For fever, diarrhea, sores
Conyza scabrida	*ent*-19-Acetoxy-15,16-epoxy-5,10-seco-1,3,5,19*Z*,13(16),14-clerodapentaen-18-oic acid (=19-acetoxyseco-nidoresedaic acid), 5α-hydroxy-5,10β-dihydro pintzianic acid, 5α-hydroxy-1,2-dehydro-5,10-dihydropintzianic acid	
Conyza stricta	Labda-8(17),13-dien-15-dioic acid butenolide, 16-hydroxylabda-8(17), 13-dien-15,19-dioic acid butenolide, *ent*-15,16-epoxy-5,10-seco-1,3,5(19),13(16),14-clerodapentaen-18-oic acid (=strictic acid), 12*R*-hydroxystrictic acid	Anti-inflammatory, anthelmintic, diuretic For cancerous, liver, and urinary disorders, chickenpox, diarrhea, dysentery, small pox, stomach ulcers
Conyza welwitschii	15,16-Epoxy-5,10-seco-1,3,5(19),13(16),14-clerodapentaene-17,18-dioic acid, 12*R*-hydroxystrictic acid, 17α-hydroxy-12β,17-epoxystrictic acid	
Copaifera cearensis	Epuruic acid, cativic acid, kolavenic acid, crolechinic acid, haplociliatic acid, labdanolic acid, patagonic acid, (-)-hardwickiic acid	NO production inhibitory
Copaifera coulteri	15,16-Epoxy-2-hydroxy-3,13(16),14-clerodatrien-18-oic acid (=2β-hydroxyhardwickiic acid	
Copaifera duckei	(-)-Hardwickiic acid, kaur-16-en-19-oic acid (=kaurenic acid), *ent*-polyalthic acid	Anti-inflammatory, antinociceptive, antiproliferative, antitrypanosomal
Copaifera langsdorffii	(-)-Hardwickiic acid , kaur-16-en-19-oic acid (=kaurenoic acid), *ent*-8(17)-13*E*-labdadien-15-oic acid (=copalic acid), acetoxycopalic acid (=3α-alepterolic acid acetate, *ent*-agathic acid, hydroxycopalic acid (=3α-alepterolic acid), 3-hydroxy-14,15-dinorlabd-8(17)-en-13-one, kaur-16-ene, manool (**34**), sclareol (**22**)	Food additive, flavoring agent in foods and beverages Anodyne, antacid, antibacterial, antifungal, anti-inflammatory, antileishmanial, antimicrobial, antiproliferative, antipsoriatis, astringent, astroprotective, cytostatic, demulcent, digestive, disinfectant, diuretic, expectorant, insecticidal, laxative, smooth muscle relaxant, vermifuge, vulnerary, wound healing
Copaifera lucens	Copalic acid, *ent*-polyalthic acid	Antibacterial, antileishmanial

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Copaifera martii	Kovalenic acid, kaur-16-en-19-oic acid (=kaurenic acid)	Antibacterial, anticancer (cervical carcinoma), anti-inflammatory, antileishmanial
Copaifera multijuga	Copalic acid, 3-hydroxycolalic acid, acetoxycopalic acid, 18-hydroxy-8(17),13-labdadien-15-oic acid (=copaiferolic acid)	Antibacterial, antiblenorragic, anticancer, anticough, anti-inflammatory, antileishmanial, antinociceptive, antiproliferative, antirheumatic, antiseptic, antitetanis, diuretic, insecticidal, laxative
Copaifera officinalis (Copaiba balsam)	15,16-Epoxy-3,13(16),14-clerodatrien-18-oic acid (=copaic acid), (-)-hardwickiic acid, kaur-16-en-19-oic acid (=kaurenic acid), copalic acid	Food additive, flavoring agent Antibacterial, anticancer, antifungal, anti-inflammatory, antimicrobial, antileishmanial, antiproliferative, astringent, cytostatic, demulcent, digestive, disinfectant, diuretic, expectorant, laxative, vermifuge, vulnerary. topical wound healer For bronchitis, chilblains, eczema, insect bites, psoriasis, sinusitis, sores, urinary tract and reproductive system conditions such as cystitis, kidney, bladder infections, vaginal discharge and gonorrhea, and tonsillitis, tuberculosis
Copaifera paupera	Copalic acid, kaur-16-en-19-oic acid (=kaurenic acid), methyl copalate, agathic acid 15-methyl ester, agathic acid 15,19-dimethyl ester, *ent*-polyalthic acid, *ent*-pinifolic acid, methyl 3β-hydroxylabda-8(17),13-dien-15-oate, methyl 18-hydroxycopaiferolate, 14,15-bisnorlad-8(17)-en-13-one, 16β-kauran-19-oic acid	Antibacterial, antileishmanial, antiproliferative
Copaifera reticulata	14-Clerodatrien-18-oic acid (=hardwickiic acid), *ent*-kaur-16-en-19-oic acid (**47**), *ent*-8(17)-13*E*-labdadien-15-oic acid (=copalic acid), acetoxycopalic acid (=3α-alepterolic acid acetate), *ent*-agathic acid, hydroxycopalic acid (=3α-alepterolic acid), 3β-hydroxylabdan-8(17)-en-15-oic acid, *ent*-	Acaricidal, antibacterial, antinociceptive, antifungal, anti-inflammatory, antileishmanial, antiproliferative, antitrypanosomal, anxiolytic, insecticidal (mosquito larvicidal), neuroprotective

	15,16-epoxy-13(16),14-clerodadien-18-oic acid, 13*S*-7-labden-15-oic acid, *ent*-8(17),13-labdadien-15,19-dioic acid (=agathic acid)	
Copaifera scabrida	19-Acetoxy-1,2-dehydro-hautriwaic acid	
Crossopetalum gaumeri	Crossogumerin B, nimbiol	Cytotoxic (HeLa)
Crossopetalum uragoga	Salvadoriol, ferruginol, triptinin, hypolide, carnosol (**16**), carnosol diacetate	Antitumor promoting, antioxidant
Croton argyrophylloides	15,16-Epoxy-4-hydroxy-13(16),14-clerodadiene-3,12-dione, 15-oxo-kaur-16-en-18-oic acid	Antispasmodic, cytotoxic (HL-60: leukemia; MPAMB-435: melanoma; SF-295: glioblastoma; HCF8: colon cancer cell lines)
Croton cajucara	Cajucarin A, (*E*)-cajucarin B, (*Z*)-cajucarin B, *ent*-(4α*H*,5β-12β*H*)-5,16-epoxy-19-nor-2-oxo-13(16),14-clerodadien-20,12-olide (=(*E*)-crotonin), (*E*)-dehydrocrotonin	Analgesic, antidiabetes, antihypercholesterolemic, antileishmanial, antinociceptive, antiulcerogenic, gastroprotective, cytotoxic for Ehrlich carcinoma, human K52 leukemia and peripheral blood mononuclear cell, antilipotropic, NO production inhibitory For diarrhea, liver inflammation
Croton californicus	(-)-Methyl barbascoate, *ent*-(5β,12α*H*)-15,16-epoxy-3,13 (16),14-clerodatrien-17,12-olid-18-oic acid (=barbasoic acid)	Antimalarial For rheumatic pain Stupefy fish
Croton campestrs	*ent*-15,16-Epoxy-20-hydroxy-2-oxocleroda-3,13(16),14-triene (=velamolone), *ent*-15,16-epoxy-2-oxocleroda-3,13 (16),14-triene (=velamone)	
Croton caudatus	Crotoncaudin, *ent*-8*S*,10*R*-15,16-epoxy-19-norcleroda-4,11,13(16),14-tetraene-18,6α:20,12-diolide (=isocrotocaudin), teucvidin, *ent*-(5β,12α*H*)-15,16-epoxy-19-nor-4,11, 13(16),14-clerodatetraene-18,6α:20,12-diolide (= 3,13(16),14-clerodatrien-17,12-olid-18-oic acid), crotocaudin, isocrotocaudin, crotusins A-C	Cytotoxic (A549, Hela, Hep G2, MPA-MB 231, MCF-7 human tumor cell lines) For colds, diuretic, fever, hypothermic, hypotensive, insecticidal, smooth muscle relaxant, stomach disorders, toxicity assessment
Croton caudatus var. *tometosus*	Crotontomentosins A-F, *ent*-15,16-epoxyhalim-5(10),12 (16),14-trien-18-oic acid, margocin, pomiferin D, E, 15-hydroxy-7-oxoabieta-8,11,13-triene	Cytotoxic (A549, Hela, Hep G2, MDA-MB-231 cancer cell lines)

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Croton corylifolius	Corylifuran, *ent*-(4α*H*,12α-)-15,16-epoxy-3-oxo-13(16),14-clerodadien-20,12-olide-18,19-dioic acid (=corylifuran)	
Croton crassifolius	Crassifolin A-G, spiro[furan-3-(2*H*),1'(2'*H*)-naphthalen)]-5'-carboxylic acid, chettaphanin, penduliflaworosin, 1,4-methano-3-benzoxepin-2(H)-one, isoteucvin, teucvin, crassifolin A-D, J-O, clerod-5(10)-ene-19,6β; 20,12-diolide, chettaphanin I, chettaphanin II, crassifolusin A	Anti-Herpes simplex, antiangiogenic
Croton damayeshu	Crodamoids A-J, 13-*O*-(2-methylbutyryl)-4-deoxy-4α-phorbol, 12-*O*-tigloyl-4-deoxy-4α-phorobol-13-acetate, 12-*O*-tigloyl-4-deoxy-4α-phorbol-13-2-methylbutyrate, 12-*O*-angeloyl-4-deoxy-4α-phorbol-13,2-methybutyrate, 4α-deoxyphorbol-12-tiglate-13-isobutyrate	Antiangiogenic, cytotoxic (A549: lung carcinoma; HL-60: human acute myeloid leukemia cell lines)
Croton draco	12,10:15,16-Diepoxy-2-hydroxy-20-oxocleroda-3,13(16),14-trien-18-oic acid, draconin, 15,16-dihydroxy-18-oxocleroda-3,13-diene, 9-acetoxy-vomifoliol, 9(11)-dehydrokaurenic acid, hardwickiic acid	Antitumoral For cough, cut, diarrhea, flu, herpes infections, open sores, oral sores, stomach ulcers, wound healing
Croton eleuteria	*ent*-15,16-Epoxy-7β-acetoxy-3β,4α,12*S*-trihydroxy-13(16),14-clerodadien-20-al (=cascarillin), *ent*-4α*H*-5,16-epoxy-13(16),14-clerodadien-2-one, cascarillin A, C, E-I, eluterins A-J	Antifungal, gastric acid secretion For digestion
Croton haumanianus	Crotocorylifuran, *ent*-15,16-epoxy-3(16),4,14-clerodatrien-20,12-olide 18,19-dioic acid, dimethyl ester, crotocorylifuran, crotohaumanoxide	Edible (stem bark, leaves, fruits) Aphrodisiac, antiepileptic antihypertensive For abortion prevention, blennoragy, constipation, diuretic, edema of the legs and abscesses, epilepsy, gastric diseases, gonorrhea, headache, pain, heartburn, hernia, hypertension, purgative, rheumatism, and painful urination, urinary infections, worm

Croton joufra	Plaunol A, C, swassin	Histaminergic effect, smooth muscle stimulant
Croton lacciferus	*ent*-Kaur-15-en-17-ol, *ent*-kaur-15-en-17-hydroxy-3β-yl acetate, *ent*-kaur-3β,16β,17-triol, *ent*-kauran-16β,17-diol, *ent*-15-kaurene-3β,17-diol	Antifungal, colds, dysentery, fever, skin diseases and lung diseases, tuberculosis
Croton laui	Crotonolide A (**116**), B-J, isocrotonolide B-D, 12-deoxycrotonolide H	Cytotoxic (HL-60, P-388), antimicrobial
Croton lechleri	Korberin A, korberin B, bincatriol, crolechinol	Antibacterial, antifungal, antioxidant, antitumoral, antiviral, CNS depressant, crown gall tumor inhibitory, cytotoxic (CA-9KB), wound healing
Croton lucidus	15,16-Epoxy-19-nor-2-oxo-13(16),14-clerodadien-20,12-olide (=crotonin)	
Croton macrostachys	Crotomacrine	Antibacterial, antifungal, early antigen viral induction stimulation, anti-inflammatory, fish poison, insecticide, laxative, mitogenic
Croton mauritianus	2-*O*-Decanoylphorbol-13-acetate, 12-*O*-decanoyl-7-hydroxy-peroxyphorbol-5-ene-13-acetate	Cytotoxic (chikungunya virus-induced cell death)
Croton megalocarpus	15,16-Epoxy-3,4-dihydroxy-12-oxo-13(16)14-clerodadien-17-oic acid methyl ester (=chiromodine)	Early antigen viral induction stimulation, Epstein-Barr virus activation, mitogenic, molluscicidal
Croton nepetaefolius	1,4-Dihydroxy-2*E*,6*E*, 12*E*-casbatrien-5-one	Antimicrobial
Croton niveus	*ent*-8(17),13-Labdadien-16,15-olid-18-oic acid (=nivenolide)	
Croton nublytatus	15,16-Epoxy-3,8(17), 13(16),14-clerodatetraene-18,19:20,12-diolide (=plaunolide)	
Croton oblongifolius (=*C. roxburghii)*	Labda-7,12*E*,14-triene-17-al, 17-hydroxylabda-7,12(*E*),14-triene, 17-acetoxylabda-7,12*E*, 14-triene, 15-hydroxylabda-7,13*E*-diene-17,12-olide, labda-7,13*E*-diene-17,12-olide, 12,17-dihydroxylabda-7,13*E*-diene	Antimicrobial, antispasmodic, hepatoprotective, histaminerigic, hypotensive, smooth muscle stimulant, tonic For fever, flat worms, dysentery, dysmenorrhea, dyspepsia, infertility, purgative, snake poisoning, wounds

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Croton schiedeanus	12*R*-12-Hydroxy-cascarillone, (*E*)-dehydrocrotonin, (Z)-dehydrocrotonin, 5β-hydroxy-(*Z*)-dehydro-crotonin, methyl (-)-16-hydroxy-19-nor-2-oxo-*Z*-cleroda-3,13-dien-15,16-olide-20-oate, (-)-12,16-dihydroxy-15,16-olide-*Z*-cleroda-3,13-dien-15-oic acid, floridolide A, (+)-15-methoxyfloridolide A, haplopappic acid, (+)-15-hydroxy-*cis*-cleroda-3,13-dien-15-oic acid	Antihypertensive, vasodilator effect
Croton sonderianus	*ent*-(10β,12α*H*)-3,13(16), 14-Clerodatrien-20,12-olid-18-oic acid methyl ester (=sonderianin), 15,16-epoxy-12-hydroxy-3,13(16)14-clerodatrien-18-oic acid (=12-hydroxyhardwickiic acid)	Antimicrobial
Croton sublyratus	2,19-Dihydroxy-3,8(17), 13(16),14-clerodatetraene-18,6:20,12-diolide, (=plaunol C), plaunol A, B, D (=plaunol), plaunol E (=plaunol acetate)	Analgesic antibacterial, antifungal, anticholinesterase, antiulcerogenic, hemorrhagic, toxicity assessment
Croton tonkinensis	*ent*-1α-Acetoxy-7β,14α-dihydroxy-kaur-16-ene-15-one, crotonkinin A, crotonkinins C-J, (16*S*)-*ent*-18-acetoxy-7β-hydroxykaur-15-one, *ent*-18-acetoxy-7α-hydroxykaur-16-en-15-one, *ent*-14β-acetoxy-7α-hydroxykaur-16-en-15-one, *ent*-7α,14β-dihydroxykaur-16-en-15-one, crotonkinensin A, *ent*-7β,18-dihydroxykaur-1-en-15-one, *ent*-15-oxokaur-16-en-18-oic acid, *ent*-18-acetoxykaur-16-en-15-one, *ent*-7β-hydroxykaur-16-en-15-one, *ent*-14β-hydroxykaur-16-en-15-one, *ent*-11α-acetoxykaur-16-en-18-oic acid, *ent*-18-hydroxykaur-16-en-15-one, *ent*-18-acetoxy-7α-hydroxykaur-6-en-15-one, *ent*-18-acetoxy-7,14β-dihydroxykaur-16-en-15-one	Elastase release, superoxide radical inhibition
Croton urucurana	Sonderianin, methyl 12-*epi*-barbascoate	Antidiarrheal
Croton zambesocus	Crotozambefurans A-C, crotocorylifuran	Antibacterial, antifungal, antiviral, protease inhibitory

Croton zambesocus	*ent*-18-Hydroxy-trachyloban-3-one	NO production inhibition
Cryptomeria japonica	8,(14),15-Isopimaradien-18-ol (=isopimarinol), 11-hydroxy-12-methoxy-8,11,13-abietatrien-7-one (=cryptojaponol), ferruginol, 7-oxoferruginol, xanthoperol, sandaracopimaradien-18-ol, 15-kaurene, sandaracopimaric acid, sandaracopimaradiene-3β-ol, sugiol, secoferruginol, sandaracopimaric acid, sandaracopimaradiene-3β-ol	Antimicrobial, immune protective For depurative, gonorrhea
Cunninghamia lanceolata	Lanceolatanol hydroperoxide, epilanceolatanol hydroperoxide, lanceolatanoic acid hydroperoxide, epilanceolatonic acid hydroperoxide, lanceolatanol, lanceolatanoic acid, 11-acetoxylanceolatanoic acid methyl ester	Cytotoxic (PC-3: human prostate cancer cell line)
Cupressus sempervirens	6-Deoxytaxodione (=11-hydroxy-7, 9(11), 13-abietatrien-12-one), taxodione, ferruginol, sugiol, manool (**34**), 1,3-dioxoferruginyl methyl ether, sempervirol, 13-hydroxy-8,11,13-totaratrien-1,3-dione (=1,3-dioxototarol)	Antimicrobial, antileishmanial antiparasitic
Cupressus torulosa	Manool (**34**)	Antiseptic For inflammatory wound healing
Curcuma amada (*mango ginger*)	12β-Hydroxy-15-norlabda-8(17),13(14)-dien-16-oic acid, (*E*)-15-ethoxy-15-methoxylabda-8(17),12-dien-16-al, (*E*)-15α-ethoxy-14α-hydroxylabda-8(17),12-dien-16-olide, 15-ethoxy-12β-hydroxylabda-8(17), 13(14)-dien-16,15-olide, (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one, coronarin C ethyl ester, coronarin D methyl ester, (*E*)-15,15-diethoxylabda-8(17),12-dien-16-al, (*E*)-labda-8(17),12-diene-15,16-dial (**29**), (*E*)-14-hydroxy-15-nor-labda-8(17),12-dien-16-ol, (*E*)-labda-8(17),12,14-trien-15,16-olide, zarumine, methyl 12*E*,16-epxolabda-8(17), 12-dien-15-one, 16-oxolabda-8(17),12-dien-15,11-olide, (*E*)-14,15,16-trinorlabda-8(17),11-dien-13-oic acid (16), (*E*)-labda-8(17),12-dien-15-ol-17-al, (*E*)-14-hydroxy-15-norlabda-8(17),12-dien-16-al	Food (dippig vegetable) Analgesic, anticancer, antifungal, antimicrobial, antioxidant, antiplatelet, antitubecular, antiulcer, anti-inflammatory, CNS depressant, cytotoxic (A549: lung; HeLa: cervical; MCF7:human breast cancer; PANC-1 and PSN-1, pancreatic cancer cell lines), larvicidal, insecticidal For cough, inflammation, rheumatisms

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Curcuma domestica (=*C. longa*)	(*E*)-Labda-8(17),12-diene-15,16-dial (**29**)	Foods, food additive, beverage (herbal tea) α-Amylase inhibitory, antiallergic, anti-Alzheimer's disease, antiarthritic, anticarcinogenic, antidiabetes, antifungal, anti-inflammatory, antiobesity, antioxidant, antiviral, hypoglycemic, hypolipidemic, neurotoxin inhibitory
Curcuma heyneana	(*E*)-15,16-Bisnorlabda-8(17),11-dien-13-one, (*E*)-labda-8(17),12-dien-15,16-dial (**29**)	Anti-inflammatory, antimicrobial, antioxidant, cancer prevention For anthelmintic in skin scrub, body sliming, diarrhea, wound healing
Curcuma mangga	(*E*)-15,16-Dinorlabda-8(17),11-dien-13-one, communic acid, labda-8(17),13-diene-15-oic acid (copallic acid) (**30**), 14,15,16-trinorlabdan-8,12-diol, (*E*)-labda-8(17),12-diene-15,16-dial (**29**), zerumin A (**69**), B	Foods, beverage (herbal tea) Analgesic, antiallergic, antioxidant, NO production inhibitory, lipid peroxidation, antiproliferative, antimicrobial, antitumor, apoptotic, cytotoxic (A549, Ca Ski, HT-29, MRC-5, KB, cancer cell lines)
Curcuma zedoaria	Curcuminol	Food, beverage (herbal tea) Antiamoebic, antifungal, antimicrobial, antiulcer, digenetic, hepatoprotective For cold, infection, diarrhea, leukoderma, leucorrhea, menstruation, stagnation, stomachache, toothache, tuberculosis, vomiting
Cussonia racemose	Cussoracosides A (**19i**)-F, β-D-glucopyranosyl *ent*-16β,17-dihydroxykauran-19-oate, paniculoside IV	Antispasmodic For acne, diarrhea, mental diseases, malaria, rheumatism, syphilis
Cyathus africanus (mushroom)	Neocyathins K-R, three known congeners	Antineuroinflammatory, NO production inhibitory
Cyathus earlei (mushroom)	3,13-Cyathadien-11α,14β,15-triol (=cyathatriol), 3,13-cyathadien-14β-acetoxy-11α,15-diol, 3,13-cyathadien-15-acetoxy-11α,14β-diol, 3,13-cyathadien-11α,15-diacetoxy-14β-ol	Antibacterial, antifungal

Cyathus helenae (mushroom)	Cyathin A_3, B, B_2, Q, allocyathin, neoallocyathin A_4, cyahookerins A-F, (12*S*)-11α,14α-epoxy-13α,14β,15-trihydroxy-cyath-3-ene, (12*R*)-11α,14α-epoxy-13α,14β,-15-trihydroxycyath-3-ene, cyathin E, erinacol, cyathatriol, 4-oxo-cyatha-3,12-diene	Antibacterial, neurite out growth promoting, NO production inhibitory
Cymbopogon citratus	α-Camphorene (**136**)	Flavoring agent in foods, drinks and in perfumes, baked goods and beverage (herbal tea) Analgesic, antimicrobial, antifungal, anti-inflammatory, antimutagenic, carminative, sedative For arthritis, febrifuge, digestion problems, cramping flatulence, immunity booster, mosquito repellent
Cystoseira abiesmarina	Cystoazorols	Antioxidant
Cystoseira myrica	Dictyone acetate, dictyol F monoacetate, isodictytriol monoacetate, cystoseirol monoacetate	Cytotoxic (KA3IT: murine cancer cell line)
Cystoseira sp. (algae)	Meroterpenoids	Antibacterial, anticancer., antidiabetes, antifungal, anti-inflammatory, antioxidant, antiparasitic, cholinesterase inhibitor, cytotoxic
Cystoseira usneoides	Cystodiones A-F	
Dacrydium biforme	Labda-8(17),14-dien-13-ol (=manool) (**34**)	
Dacrydium cupressinum	Podocarpic acid, totarol	Anti-influenza, anti-LAV, astringent
Daphne genkwa	Genkwadaphnin	Anti-influenza, antiviral [hepatitis B and C virus (HBC|V, HCV); HIV]
	Genkwadanes	Cytotoxic (HT-1080 cell line)
Dicraropteris dichotoma	18-Hydroxyaylthonic acid (**117**), 18-oxo-aythonic acid	Anti-HIV
Dictyota acutiloba (brown algae)	Acutilols A, B, acutilol A acetate, dictyolene	Feeding deterrent
Dictyota bartayresii	8β-Hydroxypachydictyol A	Antiviral, cytotoxic
Dictyota binghamiae	Dictyoxide A, dictyotriol A diacetate	
Dictyota caribaea	Dictyol B acetate, pachydictiol A, isopachydictyol A	Antialgal, antifouling, antiherbivory, antiproliferative, antithrombotic, cytotoxic

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Dictyota cervicornis	8,14β-Epoxy-7β,8-dihydroxy-8,9-seco-1,15-dolasten-9-one	Antifeedant, antifouling, antimalarial, antiviral, enzyme inhibitory
Dictyota ciliolata	Dictyol B acetate, pachydictyol A, xenicane diterpenoids	Antialgal, antimicrobial, antifouling, antifungal, antiproliferative, antithrombotic, cytotoxic
Dictyota crenulata	Dictyodial	Antibiotic, antifouling, antifungal
Dictyota dentata	Dictyol C, H (**119**)	Antioxidant, antitumor, protection for DNA damage
Dictyota dichotoma	Dictyol A (**118**), B-F, J, dictyol B acetate, dictyol-D 2β-acetate, dictyol I acetate, pachydictyol A-C, isopachydictyol A, (*Z*)-pachydictyol, (*E*)-pachydictyol, 8α-dihydroxypachydictyol A, 8β-hydroxypachydictyol A, 3,4-epoxy-13-hydroxypachdidctyol A, dictyoxide, dictytriol, dictyone, dictyone acetate, dictyotadiol, dictyohydroperoxide, isopachydictyolal, dictyotins A-C, *ent*-ergorgiaene, dolabellatrienol, amijiol, amijiol acetate, dichotenol B, dichotone, acetyldictyolal, dictyotalides A, B, nordicytotalide, 4-acetoxydictyolactone, crenulacetal C, acetoxycrenulide, sanadaol, 13-epidictyol, dictyone, dictyriol, isodictyriol, dictyriene A, dictytriene, epoxypachydictyol A (**120**), 9α,15-epoxy-7(17)-fusicoccene (=epoxydictyotene), norxenicanes 3*E*,7*E*,18-dolabellatrien-14-one (**31**), secodolastanes, dictyoxetanes (Group 2), xenicanes, 4,18-dihydroxy-1(9),6,13-xenicatrien-19-al (**33**)	Food, animal feed, make beer, frozen food, fruit juices, pastries, ice cream, jellies, in meat and flavor sauces, milk shakes, salad dressings Acyltransferase and diacylglycerol inhibitory, algicidal, antibacterial, antifouling, antifungal, antiherbivory, antioxidant, antithrombotic, antitumor, antiviral, cytotoxic, fish antifeedant, protection of DNA damage, pesticide
Dictyota divaricata	Dictyol C, H (**119**), 2-hydroxydictyoxide, dilophol, 3β-hydroxydilophol, 7β-acetoxy-1(15),8-dolastadiene-14-ol	Antifouling, antioxidant, antitumor, protection for DNA damage
Dictyota flabellata	Dictyodial	Antibiotic, antifungal

Dictyota linearis	Dictyodial, 4α-acetyldictyodial, 11(15),8,13-dolastatriene-4-diol, 11(15),8-dolastadiene-2,14-diol (=amijitrienol), dolastanes, 1(15),8-dolastadiene-2β, 14β-diol (=isoamijiol) (**32**)	Antibacterial, antifungal
Dictyota menstrualis	Pachydictyol A, isopachydictyol, dictyol A, 6*R*-6-hydroxydichototomo-3,4-diene-1,17 isoamijiol -dial, 6*R*-acetoxydicitotomo-3,4-diene-1,17-dial	Antithrombotic, antifouling, cytotoxic, anti-HIV-1, antithrombin, antifeedant, antiretroviral
Dictyota mertensii	Dictyol H (**119**), epoxypachydictyol A (**120**)	
Dictyota pfaffii	Dolabelladienols A, B, dolabellanetriol, dolabelladiene, 8-hydroxy-2,6-dolabelladiene	Antifeedant, antiviral, antimalarial, antiviral, anti-HSV-1, antithrombin antifeedant, pasture weeds inhibitory
Dictyota plectens	8α,11-Dihydroxypachydictyol A, 8β-hydroxypachydictyol A, 9α-hydroxydictyol, isodictyol E, 3β-acetoxydilophol, acetoxypachydiol, 4α-hydroxypachylactone	Anti-inflammatory, antimalarial, antioxidant, antiviral, cytotoxic
Dictyota prolificans	1(9)*E*,6*E*12*E*,14-xenoicatetraene-17,18:19,18-diolide, 12,15-didehydro-1,9*E*,6*E*-13-xenoicatriene-17,18:19,18-diolide	
Dictyota sp.	Xenicane-type diterpenoids, 18-hydroxydolabella-3,7-dien-2-one, 3,4-epoxy-2α,18-dihydroxydolabella-7-ene, 3,4-epoxy-18-hydroxydolabella-7-ene-3β-hydroxydilophol, dictyol C, E, hydroxycrenulide, 9-acetoxy-15-hydroxy-1,6-dolabelladiene, hydroxyacetyldictyolal, 4,8-dihydroxydictyo-lactone, 8α,11-dihydroxypachydictyol	Antifoulants, cytotoxic (NCI-H189)
Dictyota spinulosa	Hydroxydictyodial	Antibiotic, antifeedant
Dictyota valubilis	Pachydictyol	Antifouling, antithrombotic cytotoxic
Dilophus dentata	Dictyol H	
Dilophus fasciola	Dictyol C, E, dolabellanes, neodictyolactone, sanadaol, 2*E*,4βH,7*E*-dolabelldiene-5β,6β,10α,18-tetrol, 7,18-fascioladien-17-al	Acyltransferase inhibitory, algicidal, antifouling, cytotoxic, ichthyologic, phytotoxic

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Dilophus ligulatus	Dictyol E, dictyoxide	Acyltransferase inhibitory
	Epoxyoxodolabelladiene, acetyldictyolal, dictyotalide, neodictyolactone, dictyolactone, 6,13-xenicatrien-19-al (=fukurinolal), pachylactone, acetoxycrenulide, acetylcoriacenone, isoacetylcoriacenone, grenuladial, dilophol, dictyoxide	Antifungal, antimicrobial, cytotoxic
Dilophus marginatus	Secospatane-types, cubebanes, 13,17-spatadien-10β-ol	Antimicrobial Feeding deterrent
Dilophus mediterraneus	Dictyol C	Antifouling
	Dictyol E	Acyltransferase inhibitory
	Dictyodial, sanadaol	Antibacterial, algicidal
Dilophus okamurai	Secospatane-types 15, 17, 18-dictyolactone dikamural, pachydictyol A, 6,13-xenicatrien-19-al (=fukurinolal), cubebane type, dictyoterpenoids A, B, 4-hydroxy-18-acetoxy-1(9),6,13-xenicatrien-19-al (=fukuinolal)	Antimicrobial, cytotoxic, feeding deterrent
Dilophus prolificans	Dilopholone, 7α-acetoxydilopholone, (1, 7β-acetoxydilopholone, epoxydilopholone	
Dilophus spiralis	Pachydictyol A, isopachydictyol A, dictyoxide, dolabellanes, neodictyolactone, acetyldictyolal, pachylactone, dilospirane B	Antibacterial, antithrombotic, antifouling, antitumor, feeding deterrent, cytotoxic
Dilophus ugandensis	Dictyol E	Acyltransferase inhibitory
	Dilophic acid	Antibacterial

Dioscorea bulbifera	Diosbulbin N (**121**) diosbulbins A-D, F, G, O, P	Food Analgesic, antifungal, anti-inflammatory, antimicrobial, antioxidant, antitumor, gastroprotective functions
	8β,12α:15,16-Diepoxy-9-*nor*-13(16),14-clerodadiene-16,6β:18,2α-diolide (=diosbulbin B), diosbulbioside F, diosbulbin E	Anti-inflammatory, cytotoxic (A-549, HL-60, MCF-7, SMMC-7721, SW-480 human cancer cell lines), hepatoprotective For leprosy, tumor
Dioscoreaphyllum cumminsii	Isocolumbin	
Dodonaea angustifolia	2-Hydroxy-15,16-epoxycleroda-3,13(16),14-trien-18-oic acid, *ent*-16-hydroxylabdan-3α,8β-dihydroxy,13(14)-en-15,16-olide	Antibacterial, hypotensive, cardiac depressant, coronary-constricting, phagocytosis enhancing, analgesic, molluscicidal, intestinal smooth muscle contraction, anodyne, antipruritic, astringent, diaphoretic, febrifuge, odontalgic, vulnerary For fevers, malaria, swellings, wounds A decoction: for digestive system disorders, including indigestion, ulcers, diarrhea, and constipation
	Dodonic acid (**122**), 2β-hydroxyhardwickiic acid, *ent*-3β,8α-dihydroxy-15,16-epoxy-13(16),14-labdadiene, hautriwaic acid, hautriwaic acid lactone, neoclerodan-3,13-dien-16,15:18.19-diolide, 15α-methoxy-neoclerodan,3,13-dien-16,15:18,19-diolide, 15β-methoxyneoclerodan-2,13-dien-16,15:18,19-diolide	Antifungal, antimicrobial For tuberculosis, pneumonia
Dodonaea attenuata	*ent*-17-Acetoxy-15,16-epoxy-19-hydroxy-3,13(16),14-clerodatrien-18-oic acid	
Dodonaea polyandra	polyandric acid A, B, 13,17-epoxy-13-methyl-15-oxo-labda-7-ene (**123**), 17-hydroxy-13-methyl-labda-7,13*Z*-dien-15-oic acid, 13-methyl-17-oxo-labda-7,13*Z*-dien-15-oic acid, 15,16-epoxy-3β-hydroxy-labda-7,13(16),14-triene	Anti-inflammatory, toothache

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Dodonaea viscosa	`*ent*-15,16-Epoxy-3β,8α-dihydroxylabda-13(16),14-diene, *ent*-15ξ-ethoxylabdan-3β,8α-dihydroxy-13(14)-en-15,16-olide (**124**), *ent*-16ξ-hydroxy-labdan-3β,8α-dihydroxy-13(14)-en-15,16-olide, 8α-hydroxy-3-*O*-β-glucopyranosyl *ent*-labda-13-en-15,16-olide	Antiviral (anti-herpes) For fever, hepatoprotective, hyperpigmentation-associated diseases, inflammation, insecticide, rheumatism, pain
	Hautriwaic acid, 2α-hydroxyhautriwaic methyl ester, *ent*-15,16-epoxy-9αH-labda-13(16),14-diene-3β,8α-diol, dehydro hautriwaic acid, methyl dodonates A-C, donolide, *ent*-15,16-epoxy-6-hydroxy-3,13(16),14-clerodatrien-18-oic acid (=dodonic acid)	Cytotoxic (THP-1 cell line)
Elettaria cardamomum	8(17),12-Labdadiene-15,16-dial (**29**)	Food flavor, cookies, source, liqueurs, luxury spices Antifungal, antimicrobial, antiviral, carminative, stomachic, tonic For asthma, colic, constipation, diarrhea, diuretic, dyspepsia, epilepsy, hypertension, muscle pain
Elodea canadensis	9α,13α-Epidioxy-8(14)-abieten-18-oic acid	
Elsholtzia bodinieri	Hinokiol, ludongnin, sandaracopimar-15-en-8β,12β-diol, 6-hydroxy-(-)-hardwickiic acid 2'-β-*O*-glucopyranosyl-benzyl ester, 6,7-dihydroxy-(-)-hardwickiic acid 2'-β-O-glucopyranosyl-benzyl ester	Analgesic, antibacterial, anti-HCV, anti-inflammatory, antipyretic, sedative For respiratory disorders
Eremophila aff. drummondii	8,16-Dihydroxyserrulat-19-oic acid, 7,8,16-trihydroxyserrulat-19-oic acid, 7,8-dihydroxy-16-caffeoyl-serrulat-19-oic acid, 7,8-dihydroxy-16-feruloyl-serrulat-19-oic acid, 7,8-dihydorxy-serrulat-14-en-19-oic acid, 7,8-	α-Glucosidase and PTP1B inhibitory, antimicrobial

	dihydroxy-16-butanoyloxyserrulat-19-oic acid, 7,8-dihydroxy-16[(3-methylbutanoyl)-oxy]-serrulat-19-oic acid, 8-hydroxyserrulat-4-en-19-oic acid, 8-hydroxy-16-acetoxyserrulat-14-en-19-oic acid, 3,7,8-trihydroxy-16-oxoserulat-14-en-19-oic acid, 3,7,8-trihydroxyserrulsat-14-en-19-oic acid, 7,8,16,18-tetrahydroxy-19-serrulatanoic acid, 7,8,16-trihydroxy-18-acetoxyserrulat-19-oic acid, 7,8,16-trihydroxyserrulat-19-oic acid	
Eremophila cuneifolia	5,14-Dihydroxy-3-visiciden-20-oic acid, 5-hydroxy-4-oxo-3-visciden-20-oic acid, 5-hydroxy-3,14-viscidadien-20-oic acid	Colds
Eremophila dempsteri	15-Hydroxy-3,7,11-cembratrien-19-oic acid	
Eremophila dicipiens	2(4),14-Decipiadiene-1,18-triol, 18-hydroxy-1(4)14-decipiadien-1-oic acid	
Eremophila duttonii	Serrulat-14-en-7,8,20-triol, serrulat-14-en-3,7,8,20-tetraol	Antibacterial, antilisterial
Eremophila falcata	8,16-Dihydroxyserrulat-14-en-19-oic acid	α-Glucosidase and PTP1B inhibitory
Eremophila flaccida	Serrulat-14-ene-8,18-diol	
Eremophila freelingii	5,15,17-Erematrien-19,16-olide (=eremolactone)	Tea, sugar substitute Antidiarrheal, antiseptic For chest pain, cold, headaches, wash for sores
Eremophila georgei	3,15-Epoxy-18-hydroxy-7,11-cembradien-19-ol	
Eremophila gibossa	8,16-Dihydroxyserrulat-14-en-19-oic acid, 8-hydroxy-16-oxoserrulat-14-en-19-oic acid	α-Glucosidase and PTP1B inhibitory
Eremophila glabra	8,16-Dihydroxyserrulat-19-oic acid	α-Glucosidase and PTP1B inhibitory
	8-Hydroxy-16-cinnamoyl serrulat-19-oic acid, 4-methylpent-3-enoylserrulat-19-oic acid	PTP1B inhibitory
Eremophila granitica	14-Serrulatene-2α,7,8,20-tetraol, 2-deoxy-14-serrulatene-7,8,20-triol	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Eremophila lucida	(3Z,7Z,11Z)-15-Hydroxycembra-3,7,11-trien-19-oic acid, 5α-hydroxyviscida-3,14-dien-20-oic acid	Antimicrobial
Eremophila microtheca	Microthecaline A (**125**)	Antimalarial (*Plasmodium falciparum*)
	2-Acetoxy-7,8-dihydroxy serrulat-14-en-19-oic acid, 3,7,8-trihydroxyserrulat-14-en-19-oic acid, 3,19-diacetoxy-8-hydroxy serrulat-14-ene	Antibacterial
Eremophila neglecta	8-Hydroxyserrulat-14-en-19-oic acid, 8,19-dihydroxyserrulat-14-ene, 8-Hydroxyserrulat-14-en-19-oic acid	Anti-inflammatory, antimicrobial
Eremophila rotundifolia	5,8-Dihydroxyzerrulat-14-en-18-al	
Eremophila serrulata	20-Acetoxy-8-hydroxyserrulat-14-en-19-oic acid, 8,20-dihydroxy-serrulat-14-en-19-oic acid, 8,20-diacetoxyserrulat-14-en-19-oic acid, dihydroxyserrulatic acid	Antimicrobial
Eremophila sturtii	3,8-Dihydroxyserrulatic acid, serrulatic acid	Antibacterial, cyclooxygenase (COX-1, COX-2) inhibitory
Eremophila virens	5,16-Dihydroxy-3-visciden-20-oic acid	Antimicrobial
Erythrophleum fordii	Erythrophlesin H	Cytotoxic (PC-3: human prostate cancer cell line)
Erythrophleum ivorense	Erythroivorensin	Antioxidant
Erythroxylon monogynum	Monogonol, hydroxymongonol, (+)-hibaene, atisirene, isoatisirene, (-)-pimaradiene, 8(14),15-pimaradiene	Food (cooked or row), fruits (pleasant flavor: for perfumery) Antimicrobial
Erythroxylum passerinum	14-*O*-Methylryanodanol	Anti-Aedes (aegypti larvae)

Espelelia schultentzii	15β-Hydroxy-16-kauren-19-oic acid (=grandiflorolic acid)	
Eugenia uniflora		Fruits: beverage (herbal tea) For bronchitis, cough, influenza, intestinal trouble
Eunicea asperula	Asperketal	
Eunicea fusca	8,10,13(15),16-Lobatetraen-18-ol (=fuscol), fuscoside E	Antifouling
Eupatorium glutinosum	15-Hydroxy-7-labden-17-oic acid, 15-acetoxy-7-labden-17-oic acid	Antimicrobial
Eupatorium obtussmum	Uasdlabdanes A-F	Antiproliferative, cytotoxic (A549, lung, HBL-100 and T-45D: breast, HeLa, cervix lung: SW1573: lung, WIDR: human colon cancer cell lines)
Eupatorium turbinatum	19,20-Epoxy-7α,12,20-trihydroxy-8,12-abietadiene-11,14-dione (=conacytone), turbinatone	
Eupatorium salvia	7-Hydroxy-8(17)-labden-15-oic acid (=salvic acid), 7-acetoxy-8(17)-labdan-15-oic acid	Antimicrobial, antiseptic
Euphorbia caducifolia	8,1,3-Epoxy-17-hydroxy-11,13(15)-abietadien-15, 12-olide (=caudicifolin)	
Euphorbia dendroides	Jatrophanes	Cytotoxic (NCI-H460/R: multidrug resistant cancer cell line)
Euphorbia falcata	Cyclomyrsinane	Cytotoxic (HeLa, MCF-7 cancer cell lines)
Euphorbia fidjiana	*ent*-13*R*-Hydroxy-3,14-dioxo-16-atisene, *ent*-3,14-dioxo-16-atisene, *ent*-13*R*:14*R*-dihydroxy-3-oxo-15-atisen-17-al, *ent*-17-hydroxy-8(14),13(15)-abietadien-16-al, *ent*-16-hydroxy-8(14)-pimarene-3,15-diene	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Euphorbia fischeriana (lang-du)	Prostratin 20-*O*-(6'-acetoxy)-β-*D*-glucopyranoside, langduin A_6, deacetyllangduin A_6, langduin A_6 13-*O*-β-*D*-glucoside	Antibacterial, anti-inflammatory, antituberculosis, cytotoxic (AGS, Hep-G2 cancer cell lines) For abdominal distension, edema, cancer, intestinal parasites, psoriasis scrofula
	Fischerosides A-C, prostratin, 12-deoxyphorbol-13,20-diacetate, 12-deoxyphorbol-13-hexadecanoate, 12-deoxyphorbaldehyde-13-hexadecanoate, langduin A, langduin D, *ent*-13*S*-hydroxy-16-atisene-2,14-dione, jolkinolide A, 17-hydroxyjolkinolide A, jolkinolide B, 17-hydroxyjolkinolide B	Anti-HIV-1 For ascites, edema, cancer
	Euphonoides A-G, fischeriabietane, 25 known analogues: *ent*-abietanes, *ent*-pimaranes, *ent*-kaurenes	Cytotoxic (C-4-2B, C-4-2B/ENZR: human prostate. HCT-15, RKO: colon; MDA-MB-231 breast cancer cell line)
	Euphorin A-H, *ent*-3β-hydroxyrosa-1(10),15-diene, *ent*-3β,19-hydroxyrosa-1(10),15-diene (=ebractenoid C), *ent*-2α,3α-dihydroxyrosa-1(10),15-diene (=yuexiandajisu F, *ent*-2,3-hydroxy-18-*nor*-rosa-1,3,5(10),15-tetraene (=ebractenoid F), fischeria A, jolkinolide B, yuexiandajisu E, *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide, *ent*-16α,17-dihydroxykauran-3-one, ingenol, ingenol 3-palmitate, ingenol 3-myristinate, *ent*-rosanes, *ent*-abietanes, *ent*-kaurane, ingenane, lathyrane	Cytotoxic (MCF-7: human breast cell line)
Euphorbia guyoniana	Guyonianins E, F.	Cytotoxic (HEK293: human embryonic kidney 293 cancer cell lines)

Euphorbia helioscopia	Jolkinolide A, 11β,12β-epoxy-jolkinolide A (=jolkinolide B), 16-hydorxypseudojolkinolide B, pesudojolkinolide A, jatrophane diterpenes, euphcopenoids A, B, known analogues	Tea substitute Antiasthmatic, anthelmintic, antibacterial, antifungal, anti-inflammatory, antiallergic, antinociceptive, antipyretic, antitumor, antiviral, cytotoxic (A549, HePG2: lung; HL-60, LA795), febrifuge, insulin secretagogue, NO production inhibitory, vasodepressors, vermifuge, skin eruptions
Euphorbia jolkini	Ingenol, jolkinolide A-F	Antifeedant, anti-respiratory, antiviral, anti-RSV, cathartic, respiratory *syncytial* virus (RSV)
Euphorbia kansuensis	Euphoanoid A	NO production inhibition
Euphorbia kansui	Ingenanes	Anti-*Nilaparvata lugens*, anti-*Tetranychus urticae*
Euphorbia lathyris	Iathyrane diterpenoids analogues	Cytotoxic (MCF-7/ADM), NO production inhibition
Euphorbia lunulata	Jatrophanes	Cytotoxic (MCF-7; NCI-H460)
Euphorbia mellifera	Euphomelliferine, euphomelliferenes A, B, ingenone, jatrophane diterpenes	Antitumor (Colo 320: adenocarcinoma cells)
Euphorbia neliscopia	Jatrophane diterpenoids, 16 known related compounds, nelicoscopinolide, euphornin	Cytotoxic (HeLa, MDAMB-231 cancer cell lines)
	Euphoscopoids A-C, jatrophanes, lathyrane	Antifeedant, cytotoxic (NCl-H1975, HepG2, MCF-2)
	Euphorhelipanes A, B	Triglyceride lowering
Euphorbia neriifolia	Ingol type: euphorantins S, T, *ent*-atisan type: euphorneroides A-D 11 known diterpenoids	Anti-HIV-1
	ent-Isopimaranes: eupnerias J-P, 3β-hydroxysandaracopimaric acid	Anti-inflammatory, anti-influenza, anti-HIV-1, NO production inhibitory For cold, cough, fever
Euphorbia osyrides	2,3,5,7,8,9,14,15-Octahydroxyjatropha-6(17),11*E*-diene	Cytotoxic (Cao-4, OVCAR-3 cancer cell lines)
Euphorbia pallasii	Pseudojolkinolide A, B, 16-hydroxypseudojolinolide B, 18-hydroxy-pseudojolkinolide B	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Euphorbia paralias	Paralianone	NO production inhibitory
Euphorbia pekinensis	Diterpenes, new abietane	Cytotoxic, anticomplementary
	Casbane-type diterpenoids: pekinenin C-F, pekinenal, pekinenin A, yuexiandajisu B	Cytotoxic (A549: lung; MGC-803: gastric; Ketr-3: kidney; MCF-7: breast; HL-60: leukemia; SW620: colon; SMMC-7721: liver; KB: nasopharyngeal cancer cell lines)
	Euphpekinensin	Cytotoxic (HO-8910: ovarian; KB: nasopharyngeal, NCL-H490: lung carcinoma, SGC7901: gastric cancer cell lines)
	Pekinenal	Cytotoxic (C4-2B, C42B/ENZR, MDA-MB-23 human prostate cancer cell lines)
	Euphopanes A-C	Cytotoxic (NCI-H460: lung, KB: nasopharyngeal, HO-8910: ovarian; SGC7901: gastric cancer cell lines)
Euphorbia piscatoria	Jolkinol D, L derivatives	Cytotoxic (EPG85-257: gastric, EPP85-181: pancreatic, HT-29 cancer cell lines)
Euphorbia plumerioides		Purgative, vermifuge vomiting inducing
Euphorbia prolifera	Myrsinol diterpenes	NO production inhibitory
Euphorbia royleana	Euphoroylean A (**126**), euphoroyleans B-H, phanginin A (**127**) (including 2 cembranes, 3 ingenanes, 2 atisane, 1 kauranes) + 22 known compounds:	Anticancer, cytotoxic (A-549, BEL7402, HCT 116, MDA-MB-231 cancer cell liens)
	3β-*O*-Angeloyl-17-tigloyloxy-20-deoxyingenol, 3β-*O*-angeloyl-17-benzoyloxy-20-deoxyingenol, *ent*-19-acetoxy-16α,17-dihydroxyatisan-3-one, *ent*-16α,17-dihydroxy-19-noratisan-3-one, *ent*-16α,17-dihydroxy-19-(2*Z*-methylbutanoyloxy)-kauran-3-one, *ent*-16α,17-dihydroxy-19-tigloyloxykauran-2-one, 1-acetoxy-*ent*-abieta-8(14),13(15)-	NO production inhibitory For inflammation, rheumatism

	dien-12α,16-olide, 18-hydroxy-*ent*-abieta-8(14),13(15)-dien-12α,16-olide, *ent*-18-nor-8(14),15-iso-pimaradiene-3β,4α,12β-triol, antiquorine A, sandaracopimaradienal, 3α,12α-dihydroxy-*ent*-8(14),15-iso-pimaradien-18-al, *ent*-13*S*-hydroxyatis-16-ene-3,14-dione, *ent*-3β,13*S*-dihydroxyatis-16-en-14-one, *ent*-3α,13*S*-dihydroxyatis-16-en-15-one, *ent*-3β,19-dihydroxykaur-16-ene, eurifoloids D, E, 7-angeloyl-12-acetoxy-8-methoxyingol, 3,7,12-triacetoxy-8-benzoylingol 3,12-diacetoxy-7-hydroxy-8-methoxyingol	
	Quamurolide C, euphorantins M, *ent*-atis-16-ene-3,14-dione, eurifoloids E, G, J, L, antiquorine A	Chemoreversal activities on $P\text{-}_{8p}$ high expressed HepG2/DOX cells
Euphorbia sogdiana	6(17)-Epoxylathyranes, lathyrane, myrsinane, tigliane	Cytotoxic (EJ-138: bladder cancer cells, Jurkat T-leukemia cell lines)
Euphorbia stacheyi	Euphstrachenols A-C, 5,15-diacetoxy-3-benzoyloxy-14-oxoathyr-6(17),12*E*-diene, 3,5,25-triacetoxy-14-oxo-lathyr-6(17),12*E*-diene, jolkinol A, B, jolkinoate I, 3β,5α,20-trihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12*E*-diene, ingenane, 20-*O*-acetyl-[3-*O*-(2'*E*,4'*Z*)-decadienoyl]ingenol, 3-*O*-(2'*E*,4'*Z*)-decadienoylingenol, (3β,12α,13a)-3,12-dihydroxypimar-7,15-dien-2-one, (5β,9β,10α)-2-hydroxypimara-1,7,15-trien-3-one, yuexiandajisu C, *ent*-(3α,5β,8α,9β,10α,12α)-3-hydroxyatis-16-en-14-one, *ent*-atis-16-ene-3,14-dione, joljinolide E, *ent*-11β-hydroxyabieta-8(14),13(15)-dien-16,12-olide, stracheyioid C (22)	Cytotoxic (HGC-27: stomach cancer, MV4-11: leukemia, H460 lung, Skvo3: murine ovarian cancer cells, BaF3: lymphocyte cancer cell line)
	Stacheyioids A-C	Cytotoxic (A-549, Hela, HePG2, MCF-7, P388 cancer cell lines)
Evenia prunastri (Oak moss: lichen)	Rimuene	Fragrances Antiseptic, emollient, expectorant, fixative

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Evodia floribunda	3,13-Clerodadien-16,15-olid-18-oic acid (=floridiolide A), floridiolide B, 15,16-dihydroxy-3,13-clerodadien-18-oic acid (=floridiodic acid)	Anticancer, antiobesity
Fibraurea chloroleuca	6-Hydroxyfibraurin	
Fibraurea tinctoria	epi-8-Hydroxycolumbin, fibaruretin A-F, epi-12-palmatoside G, floribundic ester, fibraurin, fibraurinoside, fiblecin, fibreucinoside, chasmanthin, fibrauretinoside A, epi-fibrauretinoside A	Anti-inflammatory, antimalarial, diuretic, NO production inhibitory, sedative For diabetes, dysentery, eyes and stomach diseases
Fritillaria ebeiensi	6α,7β-Dihydroxylabda-8(17),12(*E*),14-triene, 6-oxo-2-hydroxylabda-7,12(*E*),14-triene	Neuroprotective
Fuerstia africana	11,15-Dihydroxy-5,7,9(11),13-abietatetraen-12-one (=fuerstione), abietanes	Anti-*Plasmodium falciparum,* cytotoxic
Gelonium aeguoreum	17-Abietanes: gelomulide, gelomulide A-G, K-X, 6β-acetoxy-1-one-8β,14α-dihydroxy-*ent*-abieta-2(3),13(15)-dien-16,12-olide, *ent*-kauranes	Cytotoxic (A549: lung, MDA-MB231, MCF-7: breast, HepG2: liver cancer cell lines)
Gelonium multiflorum	Gelomulide A-F	
Ginkgo biloba	ginkgolides A (**11**), B (**12**), C (**13**), J, M	Food (fruit), beverage (herbal tea) Altitude sickness, anticancer, anxiety, Alzheimer's disease, blood pressure problems, dementia, depression, erectile dysfunction, fatigue, macular degeneration, neuropathy, premenstrual syndrome, schizophrenia
Globba pendula	16-Oxo-8(17),12-labdadien-15-olide	Cytotoxic (H-460, MC-7, PC-3 cancer cell lines)
Glossocarya caicicola	Clerodanes	Cytotoxic (Sf9 human carcinoma)
Glyptostrobus pensillis	Gryoensin A	Cytotoxic (K562: human chronic myeloid leukemia cell line)

Gnidia glauca	Gndiglaucin	Antibacterial, antifungal, antioxidant, mosquito larvicidal, ovicidal
Gochnatia decora	7β,15β-Dihydroxykaur-16-en-19-oic acid	NO production inhibitory, neutrophil elastase inhibitory For asthma, cough, wounds
Gochnatia glutinosa	*ent*-8(14),15-Pimaradiene-3β,19-diol, *ent*-8(14),15-pimaradiene-3β,18-diol	
Gomphostemma crinitum	Cleroda-12,17-diacetoxy-17-hydroxy-3,13*E*,(14)-dien-2-one	Edible (leaves) Antimicrobial, antimalarial
Goschnatia paniculata	*ent*-6β-15,17-Trihydroxy-3-cleroden-18-oic acid (=gochnatol), gochnatoic acid	
Goyazianthus tertrastichus (=*Symphopappus casarettoi*)	*ent*-3α,4α-Epoxy-13*E*-cleroden-2β,15-diol, kolaverol derivatives	Anticancer
Grangea maderaspatana	Gramaderins A-D, 18-carboxy-16α-hydroxycleroda-3,13-dien-16,15-olide, 15-deoxypulic acid, *ent*-14,15.16-trinor-5,10-seco-11,3-(19)-cledratriene13,18-dioic acid (=norstrictic acid), 15,16-dihydro-15-methoxy-16-oxostrictic acid, norhardwickiic acid	Analgesic, acetylcholinesterase inhibitory, anti-implantational, anti-inflammatory, antioxidant, elastase release inhibitory, cytotoxic, estrogenic, superoxide anion generation inhibitory For hepatitis herpes, mastitis, snake bites
Green Vegetables, foods, ruminant meat	Phytol, 3*RS*,7*R*,11*R*-phytanic acid	Antidepressant, antimicrobial, antinociceptive, antioxidant, anxiolytic, apoptosis inducing, autophagy, anti-inflammatory, cytotoxic, hypolipidemic, immunomodulatory
Green coffee beans	*ent*-9,16β,17-Trihydroxy-19-kauranoic acid (=cofarol), *ent*-9,16β,17-trihydroxy-19-kauranoic acid β-*D*-glucopyranosyl ester (=cofaryloside)	
Gutierrezia texana	10 *cis*-Clerodanes: 6α,18-dihydroxy-*cis*-cleroda-3,13(14)-diene-15,16-olide, 18,19-dihydroxy-*cis*-cleroda-3,13(14)-dien-15,16-olide, 18,19*R*-epoxy-19-hydroxy-3,13-clerodadien-15,16-olide	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Haldwickia pinnata	*ent*-15,16-Epoxy-3,13(16),14-cerodatrien-18-oic acid (= (-)-hardwickiic acid), kolavic, kolavenolic, kolavonic acids	
Halimeda scabra	Halmendalactone	Food
Halimeda tuna	Halmendalactone, halitunal	Food Anti-coronavirus
Haliminum viscosum	13,14,15,16-Tetranor-12-hydroxy-1(10)-*ent*-halimen-18-oic acid, 14,15,16-trinor-1(10)-*ent*-halimen-13,18-dioic acid, 3β-methoxy-8-labden-7β, 15-diol, 3,11-18-sphenolobadiene-4,13,17-triol, 13,17-diacetoxy3(11)-sphenolobadien-4-ol, trimesolanone, valparene	Neurotrophic
Haplopappus baylahuen	18-Hydroxy-labda-7,13*E*-en-15-oic acid, 18-acetoxy-labda-7,13*E*-en-15-oic acid	Antioxidant, antiseptic antispasmodic For digestive
Haplopappus ciliatus	3-Cleroden-15,18-diol (**128**), 3-clerodene-15,18-dioic acid (=haplociliatic acid) (**129**)	Antiseptic
Haplopappus remyanus	18-Acetoxy-*Z*-cleroda-3-en-15-oic acid	Beverage (herbal tea)
Haplopappus unicinatus	18-Acetoxy-*Z*-cleroda-3-en-15-oic acid	Antimicrobial, digestive stimulant For gastrointestinal infection, infected wound, wound healing
Hedychium coronarium	Coronarin E (**130**), ethoxycoronarin D, isocoronarin D, methoxycoronarin D, labdanes	NF-kB, COX-I, COX-II inhibitory, cytotoxic (A549: lung, HepG2: hepatoblastoma, HL-60: promyelocytic leukemia, HeLa: cervical, HuCCA-1: *cholangiocarcinoma*, KB: epidermoid, MDA-MB231: hormone independent breast, MOLT-3: T-lymphoblast, S102: hepatocellular, T-47D: hormone dependent breast cancer cell lines)

Hedychium coronarium	Labdanes, coronarin E (**130**)	A-549: lung, HeLa: cervical, MCF-7: breast cancer cell lines
Hedychium longipetalum	Hedylongnoids A, B, C	Cytotoxic
Hedychium spicatum	Labdanes	Cytotoxic (A-549: lung, A-431: skin, Colo-205: colon cancer, MCF-7: breast cancer cell lines)
Helianthus annuus (Sunflower)	*ent*-Kaurane-2α,16α-diol, *ent*-kaurane-15α,16α-epoxy-17-al-19-oic acid, phyllocladan-16β-ol, *ent*-atisan-16α-ol, angeloylgrandifloric acid, *ent*-kaurane-16-en-19-oic acid, *ent*-kaurane-17-hydroxy-15-en-19-oic acid, *ent*-kaurane-16β, 17-dihydroxy-19-oic acid, ciliaric acid, gibberellin A_{67} (**131**), gibberellin A_{76} (**132**)	Foods (seeds and flowers) Antioxidant, astringent, cytotoxic (HepG2, MCF-7, SF-268 cancer cell lines), expectorant, diuretic For fever, sores, spider snake bites, swellings
	ent-Kauran-16α-ol (**36**), *ent*-atisan16α-ol, *ent*-kaur-16-en-19-oic acid (**47**), thujyl *ent*-kaur-16-en-19-oate, 19-trachylobanoic acid (**133**), thujyl *ent*-trachyloban-19-oate (**134**), ent-kaur-16-en-19-al, *ent*-trachyloban-19-al, *ent*-kauran-16β-ol, *ent*-kauran-l6α-ol, *ent*-kauran-16β, l9-diol, *ent*-atisan-16α-ol, *ent*-atisan-16β-ol	Antimicrobial, insecticidal, oviposition stimulants for the banded sunflower moth (*Cochylis hospes*)
	Grandiforolic acid (**135**), kaurenic acid	Antioxidant
Helianthus atrobubens	*ent*-12,16-Dihydroxy-17-angeloyl-19-kauranoic acid	Food (seed), made into sunflower butter or used to make seed yoghurt, used in salads, margarines, or in cooking, roasted seed: coffee and drinking chocolate substitute, Beverage (herbal tea) Astringent, diuretic, expectorant For febrifuge, high fever, malaria, lung ailments, nutritive, poultice on sores, pulmonary complaints, rheumatic aches and pains, snakes and spider bites, stomachic swellings
Helianthus debilis (beach sunflower)	*ent*-15β,16β-Epoxy-7-hydroxy-19-kauranoic acid, *ent*-15-kaurene-17,19-dicarboxylic acid	Food (seed), vegetable Bronchial and other pulmonary problems, cold, diuretic, expectorant For snake bites, sunstroke

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Helianthus strumosus	*ent*-7-Oxo-8,15-pimaradien-19-oic acid, *ent*-7,15-pimaradien-19-oic acid	Roots (decoction): used to get rid of worms in both adults and children
	ent-Pinara-7,15-dien-19-oic acid, *ent*-7-oxopunara-8,15-dien-l9-oic acid	Roots (infusion): used in the treatment of lung problems
Helichrysum dendroideum	*ent*-15-Kaurene-17,19-diol, 16-kaurene-3α-acetoxy-19-oic acid, *ent*-kauren-3α-(3-methylbutenoyl)-19-oic acid, 15-beyrene-3β,19-diol (=15-stachene-3β,19-diol)	
Helichrysum foetidum	Kaurene-16-en-18-oic acid	Psychoactivity
Helichrysum formosissinum	7,13-Abietadien-12-ol	
Helichrysum heterolasium	9-geranyl-α-terpineol, 16-hellicallenal	
Helichrysum italicum	12-*O*-Tetradecanoyl-phorbol-13-acetate	Antifungal, anti-inflammatory, antimalarial Flowers/Leaves: For allergies, colds, cough, fragrance industries, gallbladder disorders and skin lover, infectious and sleeplessness
Helichrysum obconicum	Geranyl *p*-cymene	Anti-inflammatory, COX-II and lipase enzyme inhibitory, NO production inhibitory
Helichrysum oligocephalum	*epi*-Manoyl oxide	Anti-inflammatory For gastrointestinal complaints
Helichrysum refluxum	3,15-Erythroxyladien-18-oic acid	
Helichrysum species (600 species)		Antiallergic, anti-inflammatory, antimicrobial, antioxidant, antipsoriasis agents, diuretic, hepatoprotective For colds, cough, digestive problem, hysteria, infection, nervousness, respiratory complaints, wound healing
Helichrysum tenax var. *tenax*	15-Kauren-18-ol, *ent*-beyer-15-en-19-ol	Antimicrobial
Helichrysum chinosphaerum	*ent*-7,13-Abietadiene	

Hericium abietis (mushroom)	Erinacine P (=helical) (**42a**)	Food
Hericium erinaceum (=H. erinaceus) (mushroom)	Erinacine A (**39**), B (**40**), C (**41**), K, P (**42a**), Q (**42b**), R, cyatha-3, 12-diene, cyatha-3(18), 12-diene, erinacol, 11-*O*-acetyl-cyathatriol, 15-*O*-acetyl-cyathatriol, 11-O-acetyl-cyathin A_3, Four erinacine related compounds	Food, Chinese medicine Antibacterial, nerve-growth-factor (NGF) synthesis stimulatory, NGF like activity, κ-opioid receptor agonist
Hericium ramosum (mushroom)	Erinacine E (hericin), erinacine P (=helical) (**42a**)	Food Antibacterial, κ-opioid receptor agonist, NGF synthesis stimulatory
Hoffmannia strigillosa (tepecajeta)	*ent*-15-Hydroxy-3,13-clerodadien-16,15-olid-19-oic acid, 19-hydroxy-3,13-clerodadien-16,15-olide	Anti-inflammatory For stomach illness
Horminum pyrenaicum (dragon's mouth)	Horminone, 7-O-acetylhorminone, agastaquinone, 15,16-dehydro-inuloyleanol, 15,16-dehydroagastol, inuroyanol, agastol, 3-deoxyagastaquinone, 7α,12-dihydroxy-8,12-abietadiene-11,14-dione (=7α-acetoxyroyleanone)	Beverage (herbal tea)
Humulus lupulus	α-Camphorene (**136**), metacamphorene (**137**)	Beverage (herbal tea) Anticancer, antifungal, anti-inflammatory, antimicrobial, antioxidant, atherosclerosis, diuretic For anxiety disorders, appetite, bladder related problems, insomnia, nervous tension reduction, relaxant and sleep aid, urinary boosts T3 and T4 production
Hypoestes forskalei	Hypoestenonols A, B, verticillarone, hypoestenone (**59d**), deoxyhypoestenone, dehydrohypoestenone, hypoestene, fusicoplagin D, 8α,9α-epoxydeoxyhypoestenone, 8α,9α-epoxy-hypoestenone (**59e**)	East African folk medicine Anti-*Plasmodium falciparum,* anti-*Leishmania infantum,* anti-*Trypanosoma cruzi, T. brucei*
Hypoestes purpurea	Hypopurins A-D	Taiwanese folk medicine Anti-phlogistic, antipyretic, liver protective, vasorelaxant

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Hypoestes rosea	Hypoestoxide, isoroseanolone, roseanolone, roseadione	For typhoid, malaria
Hypoestes serpens	Fusicoserpenol A, dolabeserpenoic acid A, 7β-hydroxyisopimara-8,15-dien-14-one, 14α-hydroxy-iso-pimara-7,15-dien-1-one, 1β,14α-dihydroxy-iso-pimara-7,15-diene, 7β-hydroxyisopimara-8(14),15-dien-1-one, 7β-acetoxyisopimara-8(14),15-dien-1-one, serpendione	Traditional Malagasy medicine Antifungal, antihypertensive
Hypoestes verticillaris	Verticillarone	For tuberculosis, chest complaints, dry cough, pneumonia, wound healing, hypertension, gonorrhea, cancer
	13-Hydroxy-7-oxo-labda-8,14-diene	Antimalarial
Hyptis fasciculata	15β-Methoxyfaciculatin, 15α-methoxyfaciculatin, methoxyneoetaefolin	Expectorant For fever, gout
Hyptis fructicosa	12,16-Epoxy-11,14-dihydroxy-8,11,13-abietatrien-7-one (=hyptol)	Analgesic, anticonvulsive hypotensive, larvicidal, vasorelaxant
Hyptis suaveolens	13-*epi*-Dioxiabiet-8(14)-en-18-ol, 8-abietene-14α,18-diol, 8-abietene-14α-hydroxy-18-oic acid	Antimicrobial, antiplasmodial, carminative For stimulant, stomachic
Hyptis umbrosa	Umbrosone	Analgesic, anti-inflammatory For cramps and fever, gastrointestinal disorders, nasal congestion, skin infection
Icacina claessensis	*ent*-3α,20:14β,16-Diepoxy-3,14α,15α-trihydroxy-7-pimaren-19,6β-olide (=icacinol)	Food
Icacina guesfeldtii	Icacinlactone, icacintricholide, icacine, icaneine, De-N-methylicaceine	Analgesic, anticonvulsant, antimicrobial, anti-Epstein-Barr virus, anti-HSV-1, anti-HSV-2, sedative For food poisoning, constipation, malaria

Icacina trichantha	Icacinol, humirianthenolide C, 2β-hydroxyhumiriantholide, 14α-methoxy-humirianthjone, 17-hydroxyicacinol, icacenone, 7α-hydroxyicacenone, 12-hydroxyicacinlactone, 2β-hydroxyicacinlactone B (=icacinlactone H), 7α-hydroxyicacinlactone, 7β-hydroxyicacinlactone B, icacinlactone A-G, I-L, icacintrichanone, icacintrichantholide, icacine, icaneine, De-N-methylicaceine, (9β*H*)-pimarane, (9β*H*)-norpimarane	Food, soup Antiasthma, antihypertensive, antimicrobial. antioxidant, aphrodisiac, diarrheal effect, soft tumor For constipation, induction of emesis and abortion malaria, poisoning, rheumatism, toothache
Illicium jiadifengpi	Jiadifenoic acids	Antiviral (Coxsackie virus B2, B3, B4, B6)
Inula royleana	Inuroyleanol, 7-ketoroyleanone, 11,14-dihydroxy-12-methoxyabieta-8,11,13-trien-7-one, 12-hydroxyabieta-8,12-dien-7,11,14-trione (=royleanone), 6,7-dehydroroyaleanone, 12-hydroxy-8,12-abietadiene-7,11,14-trione (=7-oxo-royleanone), 11,12,14-trihydroxy-8,11,13-abietartien-7-one	Beverage (herbal tea) Insecticidal, parasiticide
Isodon amethystoides	*ent*-7β,14α-Dihydroxy-16-kauren-2,15-dione (=umbrosin B), *ent*-3α,7β,14α-trihydroxy-16-kauren-15-one (=wangzaozin A), leucamein F, glaucocalyxin A, B	Antimicrobial, antitumor, antioxidant, anticoagulative, antithrombotic, antifibrotic, antineuroinflammatory For abscesses, swollen sores, pneumonia, lung abscess, pulmonary tuberculosis
Isodon effusus (=*Rabdosia effusus*)	Effusin, *ent*-7,20-epoxy-1β,6α-7-trihydroxy-16-kauren-15-one (= effusanin A), *ent*-7,20-epoxy-1β-acetoxy-6α,7-dihydroxy-16-kauren-15-one (= effusanin B), effusanin C, D	Antibacterial
Isodon eriocalyx (=*Rabdosia eriocalyx*)	*ent*-15α-Acetoxy-3,20-epoxy-6α-hydroxy-16-kaurene-1,7-dione (=maoecrystal A), *ent*-7,20-epoxy-16-kaurene-1α,3α,6α,15α-tetra-hydroxy-19-*O*-β-D-glucopyranoside (=1α,3α,6α,15α-rabdoside 2), *ent*-19-acetoxy-7,20-epoxy-1α,3α,6α,7-tetrahydroxy-16-kauren-15-one (= maoecrystal I) neolaxiflorin A, B, eriocalyxins C−E, laxiflorins J−M, maoecrystal P, maoeriocalysins A-D	Cytotoxic, inhibitory effects on human tumor K562 and T24 cells

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Isodon excisoides	1α,7α,14β-Trihydroxy-20-acetoxy-*ent*-kaur-15-one, 1α,7α,14β,18-tetrahydroxy-20-acetoxy-*ent*-kaur-15-one, 1α-acetoxy-14β-hydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one, henryin, kamebanin, reniformin C, kamebacetal A, B, oridonin (**139**)	Cytotoxic
Isodon flavidus	Flavidusin A, B, glutinosin, *ent*-kaur-16β,17-diol, siegebeckiol, 16-hydroxyferrugiol, hinokiol, maslinnic acid, fladin A, lophanic acid	Antifungal
Isodon glutinosa	Four kaurenes, one abietane: glutinosin C	Anti-inflammatory, antitumor
Isodon glutinosa	Glutinosin C	
Isodon inflexus (=*Rabdosia inflexa*)	15-Acetyldemethyl kamebacetal A, six known kaurenes, 3α,6β-dihydroxy-7,17-dioxo-*ent*-abieta-5(16)-ene, kamebacetal A, kamebakaurin, excisanin, inflexin, inflexanin C (=1α-acetoxy-7β,18-dihydroxyabieta-9(11)-en-12-one, inflexanin D (=1α-acetoxy-18-hydoxyabita-7,9(11)-dien-12-one), inflexuside A (=1,11,13-trihydroxyabieta-8-en-7-one-1-*O*-β-D-glucopyranoside, inflexuside B (=1,11,13-trihydroxyabieta-8-en-7-one-1-*O*-(2-*O*-coumaroyl)-β-*D*-glucopyranoside	Anti-inflammatory, NF κB and NO production inhibitory
Isodon japonicus (=Rabdosia japonica, Isodon japonica)	Isodonteroenes I, II, oridonin (**139**), hebeirubescensin H, rabdternin E, F, enmein (**15**), nodosin, isodocarpin, serrin, isodonal, *ent*-3,15-dioxo-7β,14α-dihydroxy-kaur-16-ene (**138**), *ent*-7β,20-epoxy-kaur-16-ene-1β,6α,7α,14α,15α-pentanol-1-*O*-β-*D*-glucopyranoside, enmein-3-acetate, isodotrisin, epinodosin, sodoponin, eponodosinol, trichodonin, hikiokoshins A-I, *ent*-6α,17-Diacetoxy-7,20:15β,16-diepoxy-7,14α-kaurenediol, maoyerabdosin, rabdosin A-C, maoyecrystal F, rabdophyllin G	Beverage (herbal tea) Antibacterial, antifungal, anti-HBV, antiindigestion, anti-inflammatory, antimicrobial, antioxidant, antitumor, melanogenesis, cytotoxic (L1210: murine lymphoma, KB cancer cell lines) For gastrointestinal disorders, indigestion

Isodon lasiocarpus	*ent*-7,20-Epoxy-1β,6α,7,11α-tetrahydroxy-16-kauren-15-one (=ladiodonin), oridonin, lasiodin	
Isodon longitubus	Nodosin, isodocarpin, 1-acetyloridonin, diacetyloridonin	
Isodon lophanthoides	Lophanic acid, 8(17),12,14-labdatriene-19-oic acid, 11β-hydroxy-isopimara-8,15-dien-3-one	Anti-inflammatory, antimalarial, cytotoxic
Isodon parvifolius	Epinodosinol, lasiodpnin, parvifolines C-N, Z, rabdoternin G, adenolin E , lasiodonin, lushanrubescensin F, parvifoliside, effusanin A, B, E, taibaihenryiin A, shikokianin, maoyecrystal J, lasiodonin acetonide, rubescensin C, I, J, P, enanderianin P, 14β,16-dihydroxy-3α,18[1-methylethane-1,1-diyl] dioxy]-*ent*-abieta-7,15(17)-diene parvifoline AA, AB, X, Y, hebeirubescensin L, trichokaurin	Cytotoxic (A-549, HT-29, K526 cancer cell lines)
Isodon pharicus	Pharicin C, R	
Isodon rubescens	Rubesanolides A, B, 9 known kaurenes, ponicidin, xindongnin	Antiangiogenic, antibacterial, anticancer, anti-inflammatory For bacterial infections, cancer, inflammation, gastrointestinal and respiratory disorders, sore throat
	Atisane, isorubesins A-E, oridonin	Cytotoxic (A549, Jurkat, MCF-7, NB4, PC3, SHSY5Y human cancer cell lines)
Isodon sculponeta	Sculponeatin A-C	
Isodon shikokianus (=*Rabdosia shikokianus*)	Isodomedin, oridonin, shikokianin, shikokianidin	
Isodon trichocarpus	Enmein (**15**), dihydroenmein, isodocarpin, nodosin, isodotrisin, oridonin, trichokaurin, trichodonin, enmedol, enmenal, emerol, ememodin	Antitumor
Isodon umbrosus (=*I. kameba*)	Isodomedin, *ent*-1β,7β,14α-trihydroxy-kauren-15-one (=kamebanin), *ent*-2β,7β-14α)-trihydroxy-16-kauren-15-one (=mebadonin), *ent*-3α-acetoxy-7β,14α-dihydroxy-16-*ent*-kauren-15-one, *ent*-1β,7β,14α,20-tetrahydroxy-16-kauren-15-one (=kamebakaurin), kamebakaurinin, *ent*-2α,3α,7β,14α-	Beverage (herbal tea) Antimicrobial

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
	tetrahydroxy-16-auren-15-one (=leukamenin A), leukamenin B-D, rabdolatifolin rabdoumbosanin, shikoccin, shiokoccidin, *ent*-7β,14α-dihydroxy-16-kaurene-2,15-dione (=umbrosin A), umbrosin B	
Isodon xerophilus	(11β)-6,11-Dihydroxy-7,20-dioxo-*ent*-abieta-5,8(14)-dien-16-oic acid-δ-lactone (=xerophilusin R), (11β,15*S*)-6,10,17-trihydroxy-7,20-dioxo-*ent*-abieta-5,8(14)-dien-16-oic acid δ-lactone (=xerophilusin S), xerophilusin G, I, J, K, enanderianin, rosthorin A, longikaurin B, rabeoterunin D, *ent*-abieta-7-ene-3α,16,17,18-tetraol (=xerophilusin XIV) (**140**), 15,16,17-trihydroxy-3α-18-[1-methylethane-1,1-dyl]dioxy]-*ent*-abieta-7-ene (XV), 3α,15,18-trihydroxy-16,17-[(1-methylethane-1,1-diyl)]-*ent*-abieta-7-ene (=xerophilusin XVI)	Antibacterial, anti-inflammatory, antitumor, cytotoxic (K562, MKN45, HepG2 cancer cell lines)
Isodon yunnanenis	*S-trans*-8(17),12*E*,14-labdatrien-20-oic acid, *S-trans*-12*E*,14-labdadien-20,8β-olide, hinokiol	
Itoa orientalis	13-Deoxyitol A, itol A	Antifeedant
Jatropha curcas	Jatrophalactone, curcusecone A (**141**), B-E, curcusones F-J, 4-epicurcusone E, 3-dehydroxy-2-epicaniojane, jatrogrossidion, 2-epi-jatrogrossidione, rhamnofolanes,	Cytotoxic (A-549, CNS, HL-60, MCF-7: breast, NCI-H460: lung, SF-268, SMMC-7721: SW480 cell lines) For dysentery, eczema, gonorrhea, gum bleeding, ringworm, scabies, toothache
Jatropha dioica	Riolozatrione, 6-epiriolozatrione, jatrophatrione, citlalitrione, jatropholone A, B, jatrophatrione, citlalitrione, riolozatrione	Antibiotic, antifungal, antioxidant, antiviral
Jateorhiza palmata (=*Jateorhiza columba*)	2α,3α:15,16-Diepoxy-4α-hydroxy-2,13(16),14-clerodatriene-17,12:8,1-diolide, isojateorinyl glucoside, 15,16-diepoxy-4-hydroxy-2,13(16),14-clerodatriene-17,12:18,1β-diolide (columbin), 2β,3β-15,16-diepoxy-13(16),14-clerodadiene-17,12β:18-diolide-1β-*O*-β-*D*-glucopyranoside diolide (=columbinyl glucoside), 2β,3β:15,16-diepoxy-4α-hydroxy-	Herbal mixture, herbal bitters Anthelmintic, antipyretic, laxative For diarrhea, dysentery, dyspepsia, hernia, ruptures, snake bites, weak stomach, vermifuge, gastric irritability, vomiting during pregnancy

	2,13(16),14-clerodatriene-17,12β:18,1β-diolide (=jateorin), 15,16-diepoxy-4α-hydroxy-2,13(16),14-clerodatriene-17,12α:18,1β-diolide (=palmarin), chasmanthin, palmatosides A, G	
Juniperus cedrus	Isoabienol, sandaracopimaradiene, (*E*)-totarol (**180**)	Insecticides, insect repellent
Juniperus communis	15,16-Epoxy-12-hydroxy-8(17),13(16),14-labodatrien-19-oic acid, imbricatolic acid, isocupressic acid, sandaracopimaric acid, isopimaric acid, 13*S*-8(17)-labdene-15,19-dial (=junicedral), 6,7-dehydro-8,11,13-abietatrien-12α-ol (=Δ^6-dehydroferriginol, (5α)-12-hydroxy-8,11,13-abietatriene-6,7-dione, 5β,12-hydroxy-8,11,13-abietatriene-6,7-dione, 7-oxo-8,15-isopimaradien-18-oic acid	Beverage (gin)
Juniperus oxycedrus	13*S*-8(17)-Labdene-15,19-dioic acid (=mercusic acid), 8,11,13-abietatrien-7α-ol	
Juniperus phoenicea	7,13-Abietadien-3-one, , 4-epi-abietic acid (=7,13-abietadien-19-oic acid), 8,13-abietadien-19-oic acid (=4-epi-palustric acid), 7α-hydroxy-8,11,13-abietatriene-19-al	
Juniperus rigida	8,11,13-Abietatriene-7β,11,12-triol	
Juniperus savina	8,13-Abitadiene (=palustadiene), saviperone A (**142**), sabiperones B-F, juniperolide, 3β,7α-dihydroxyabieta-8,11,13-triene, labda-13*E*-ene-8α,15-diol	Abortifacient, cytotoxic (HL-60: human promyelocytic leukemia, A549: lung adenocarcinoma, MCF7: breast adenocarcinoma, HepG2: hepatocellular carcinoma, HCT116: colorectal adenocarcinoma cell lines)
Juniperus thurifera	13*E*-Labdane-8α-hydroxy-18-al, 13Z-labdane-8α-hydroxy-18-al	
Laetia thamnia	*ent*-Kaur-16-en-19-oic acid (**47**), *ent*-β-hydroxykaur-16-ene, *ent*-kaur-16-en-3α,19-diol, *ent*-17-hydroxykaur-15-en-19-oic acid, methyl *ent*-kaur-16-en-19-oate, *ent*-kaur-16-en-3α,19-diol (acetate diester)	Cytotoxic (HT29, HCT116, MCF: breast, SW480, SW620: colon, 22Rv1, LNCaP: prostate)
Laurencia glandulifera (red algae)	Neorogioltriol	NO production and COX-II inhibitory, tumor necrosis factor-α expression inhibitor

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Laurencia intricata (red algae)	Laurencianol	Food
Laurencia irieii (red algae)	Iriediol, 7-deoxyiriediol, 7-deoxy-1β,11β-epoxy-iriediol, irieol F, 10-acetoxyiriediol, 7-deoxyirieol (=irieol E), 11-deacetoxy-pinnaterpene A (=ireol C)	
Laurencia obtusa (red algae)	Laurecianol, obtusadiol	
Laurencia pinna (red algae)	Pinnaterpene A, 18-hydroxy pinnaterpene A (pinnaterene B), pinnaterpene C	
Laurencia saitoi (red algae)	Parguerol, parguerol 7-acetate, parguerol 16-acetate, parguerol 17-acetate,	Feeding-deterrent activity against young abalone and sea urchins
Lavandula angustifolia		Edible (leaves, petals, flowering tips) Analgesic, antianxiety, antibacterial, anticancer, antifungal, anti-inflammatory, antimicrobial, antioxidant, cardiovascular, hypolipidemic, neurologic/CNS and spasmolytic effects, sedative
Lavandula multifida	15,16-Dihydroxy-7,11-dioxopimar-8(9)-ene (**143**), 15*S*,16-dihydroxy-7-oxo-pimar-8,9-ene, 15,16,17-trihydroxy-7-oxo-pimar-8,9-ene, 5,16,17-trihydroxypimar-8(9)-ene, glutinosin (=15*S*,16-dihydroxy-iso-pimar-8(9)-ene)	Edible (aerial part) Antibacterial, antifungal, anti-inflammatory, antimicrobial, antioxidant For rheumatism
Lavandula officinalis	Megastigmatrienone, gibberellin A_3	Analgesic, antibacterial, antifungal, anti-inflammatory, antiseptic, wound healing
Leonotis nepetaefolia [mota, (marijuana) in Maxico]	Bis-spirolabdanes: leonepetaefolin A-E, 15-epi-leonepetaefolin A-E, methoxynepetaefolin, nepetaefolin (**144**), nepetaefuran, dubiin, leonotinin, leomotin, nepetaefuranol	Inflorescence (leaves). marijuana substitute (flowers) Antiasthmatic, antibacterial, antidiarrheal, antifungal, anti-inflammatory, antioxidative, antispasmodic, spasmolytic, tonic For burns, cough, fever, dysmenorrhea, kidney dysfunction, rheumatism, skin infections, scalds stomachache

Leonurus marrubiastrum	*ent*-5α,10β,12*S*-12α-Hydroxy-3,13-clerodadiene-15,16:18,19-diolide (=marrubiastrol), 3,13-clerodadiene-18-oic acid β-D-glucopyranosyl ester (=marbiaside), *ent*-demethylmarrubiaketone	
Leonurus sibiricus (marihuanilla)	Preleosibirone A (**145**), 13-epi-preleosibirone A, isopreleosibirone A, leosibirone A, B, 15-epi-lepsibirone B	Marijuana substitute (leaves and roots) Antibacterial, febrifuge, respiratory stimulant
Lepechinia bullata	7-Methoxy-12-hydroxy-8,12-abietadiene-11,14-dione (=7-O-methyl-horminone)	
Letharia vulpina (Lichen)	Manool (**34**), sanadaracopimaric acid (**35**)	Beverage Stop bleeding, stomach disorder, poultice for swelling
Leucas aspera	Leucasperone A-C, leucasperol A, B, leucasperoside A-C	Antifungal, antimicrobial, antinociceptive, antioxidant, antipyretic, antirheumatism, aperient, chronic, cytotoxic, diaphoretic, emmenagogue, expectorant, hepatoprotective, insecticide, prostaglandin inhibitory, psoriasis, skin eruptions. stimulant
Leucas linifolia	8(14),15-Isopimaradien-7-one-3-*O*-[α-L-rhamnopyranosyl (1→2)]-*O*-β-*D*-glucopyranoside (=linifolisoide)	
Leucas zeylanica	12-*O*-β-D-glucopyranosyl-11,16-dihydroxyabieta-8,11,13-triene (=leucasinoside), 19-*O*-β-*D*-carboxyglucopyranosyl-12-*O*-β-*D*-glucopyranosyl-11,16-dihydroxyabieta-8, 11,13-triene, 12,19-*O*-β-D-diglucopyranosyl-11,16-dihydroxyabieta-8,11,13-triene	Leaves: cooked and eaten as a pot herb Anthelmintic, diaphoretic, sedative For headache, itch, stimulant, poultice, vertigo, wound healing
Leucothoe grayana	1α,3β,5β,6,12β,14β,11α-10(20)-Grayanatoxene-3,5,6,12,14,16-hexol	
Liatris laevigata	8,15-Epoxy-3,12,16-pimaranetriol (=*ent*-3β,8β,12α,15R)-form), *ent*-5β-epoxy-12α,16-dihydroxy-3-pinanone	
Libocedrus formosana	Shonanol (=12-hydroxy-2,8,11,13-totaratetraen-1-one	
Linaria japonica	Linaridial (=3,13-clerodadiene-15,16-dial (=*ent*-5α,13*E*)-form, linarienone (=3,13-clerodadiene-15,16-dial12,15-dihydroxy-3,13-clerodaiene-3-one)	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Linaria saxatilis	4(18),13-Clerodadiene-12,15-diacetoxy-16-ol, 11,15-epoxy-15-hydroxy-4(18),12-clerodadiene-16-al	
Linum usitatissimum	Ustatissimin A	Hepatoprotective
Litophyton viridis	(1*R*,3*E*,7*E*,11*E*)-Cembratrien-15-ol (=nephthenol)	
Litsea glutinosa		Fruit Antipyretic, astringent, furunculosis For demulcent, diarrhea, dysentery, swelling
Lobophytum compactum	Lobocompactol A, B	Cytotoxic (A549: lung, HL-60: human cancer cell lines)
Lobophytum pauciflorum	Cyclolobatriene, fuscol, lobatriene	Cytotoxic (A-431: human epidermoid skin carcinoma)
Lobophytum sp.	17,18-Epoxy-8,10,13(15)-lobatriene	Oil
Lycopodium lucidulum	12,16-Epoxy-11,14-fihudroxy-5,8,11,13-abietatetraene-7-one	
Lycopus europaeus	Euroabienol (**146**), methyl 1α-acetoxy-7α,14α-dihydroxy-8,15-iso-pimaradien-18-oate, methyl 1α,14α-diacetoxy-7α-hydroxy-8,15-iso-pimaradien-18-oate, 1α,14α,18-trihydroxy-7α-acetoxy-8,15-iso-pimaradiene, methyl 1α,14α-dihydroxy-8,15-iso-pimaradiene-18-oate, 5,9-dihydroxygeranyl linalool, methyl 7α,14β-diacetoxy-1α-hydroxy-8,15-pimaradien 18-oate, methyl 1α-hydroxy-7α,14α-diacetoxy-8,15-iso-pimaradien-18-oate, methyl 1α,7α,14α-triacetoxy-8,15-iso-pimaradien-18-oate, methyl 1α,7α-diacetoxy-14α-hydroxy-8,15-iso-pimaradien-18-oate, methyl-7α,14α-diacetoxy-8,15-iso-pimaradien-18-oate, methyl 7α,14α-diacetoxy-11α-hydroxy-8,15-iso-pimaradien-18-oate, methyl 7α-acetoxy-11α,14α-dihydroxy-8,15-iso-pimaradien-18-oate, methyl 1α,7α-aeceoty-14-oxo-8,15-isopimaradien-18-oate, methyl 14α-acetoxy-7α,11α,-dihydroxy-8,15-iso-pinaradiene-18-	Antifungal, antigonadotropic, antimicrobial, antioxidant, hyperthyroidism, immunomodulatory

	oate, methyl 7α-acetoxy-11α,hydroxy-14-oxo-8,15-iso-pimaradien-18-oate, methyl 7α-acetoxy-1α,11α,14α-trihydroxy-8,15-iso-pimaradien-18-oate, 5,9-dihydroxygeranyl linalool	
Lycopus lucidus	3*S*,5*R*,10*S*-7-Oxo-8,11,13-abietatriene-3,11,12,14-tetraol-2-*O*-β-glucopyranosyl-(1→2)-β-glucopyranoside A (= lucihirtin)	Antiviral (hepatitis virus), dilate blood vessels
Macaranga tarius	*ent*-12β-Hydroxy-8(14),15-pimaradien-2-one (=macarangonol)	
Malvastrum coromandelianum	Neophytadiene, Phytol,	Analgesic, antifungal, anti-inflammatory, antimicrobial, antioxidant, antipyretic For dysentery
Manihot esculenta (cassava)	*ent*-8α,14α-Epoxy-3β-hydroxy-9(11),15-pimaradiene-2,12-dione, *ent*-8(14)-pimaradiene-3β,12β-diol, *ent*-15-beyerene-3β,12β-dihydroxy-2-one, *ent*-1β-hydroxy-3-nor-15-beyerenone-1,12-dione, *ent*-8(14),15-pimaradiene-3β,12β-diol (=yucalexin P21)	
Marchantia polymorpha	*ent*-Labda-7,13*E*-dien-15-ol	Beverage (herbal tea) antifungal, antimicrobial, antioxidant, antitrypanosomal, antiviral, diuretic muscle relaxant
Marrubium friwaldskyamum	9α,13*R*:15,16-Diepoxy-3-oxo-14-labden-19,6β-olide	
Marrubium vulgare (white horehound)	12*S*-Hydroxymarrubiin (**147**), 3-dehydro-15-methoxyvelutine C, marrubin, peregrinin, thessaline D, marrubinone B, deacetylvitexilactone, verbascoside, leucosceptoside A, martynoside, anisofolin A, ternifolin A, 9,13*S*:15,16-diepoxy-14-labden-19,6β-olide, 9,13*R*:15,16-diepoxy-14-labden-19,6β-olide (=premarrubiin), preperegrinine	Beverage (herbal tea) horehound beer Antibacterial, antifungal, antidiabetic, gastroprotective, vermifuge-respiratory-purgative For appetite, common cold, coughs, dyspepsia, indigestion, persistent cough, shortness of breath
Maytenus cuzcoina	Cuzcol, 6-dehydroxycuzcol	Antifeedant, anti-HIV17, antitumor promoting, cytotoxic, immunosuppressive, insecticidal

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Maytenus dispermus	Maytenone, 12,13-dihyroxy-8,11,13-totaratrien-7-one (=dispermone), 13-hydroxy-7,9(11),13-totaratriene-6,12-dione (=maytenoquinone)	
Microglossa pyrrhopappa	6-Hydroxyisochilolide, 6-hydorxy-incanpteroniolide, 3α,4α:15,15-diepoxy-10-oxo-9,10-seco-13(16),14-clerodadien-20,12-olide (=pyrrhopappolide)	
Microlepia marginata	*ent*-3β,15*S*,16-Trihydroxy-7-pimaren-3-one (=fumotoshidin A), *ent*-3β,15*S*,16-trihydroxy-7-pimaren-2-one, fumotoshidin B, *ent*-16β,17,18-kauranetriol, *ent*-16β,17-kauranediol-18-*O*-(4-*O*-methyl-β-*D*-glucopyranoside) (=epimicroplepin)	
Mikania alvimii	*ent*-15-Nor-14-oxo-8(17), 12*E*-labdadien-18-oic acid	
Mikania sessilifolia	*ent*-16β,17-Epoxy-kauren-19-al, *ent*-kauren-19-oic acid 2-hydroxyethyl ester	
Milleria quinqueflora	*ent*-8(14)-Pimamarene-2α,3α,15*R*,16-tetrol, *ent*-8(14)-pimamaren-15-oxo-2α,3α,16-diol	
Momordica charantia	Geranyllinalool, phytol, isophytol	Food, vegetables, beverage (herbal tea) Antidiabetes, antihypertension
Monarda brevipes	18-Acetoxy-3,13(16),14-clerodatrien-2-one	
Monarda sp.		Beverage (herbal tea) Arthritis stimulate For colds, delirium rheumatism, colic, digestive, febrifuge, flatulence, headache, indigestion, menstruation, nausea, respiratory problem, sudorific

Montanoa tomentosa	Pretomentol (**148**), pretomexanthol (**149**), prezoapatanol (**150**), tomentanol (**151**), zoapatlin (**152**), zoapatanol, montanol, tomexanthin, tomentol	Traditional herbal medicine Aphrodisiac, ejaculation, sexual stimulant
Mulium crassifolium	Isomulinic acid, mulinic acid	
Myroxylon pereirae (Peru balsam)		Foods, hygiene products, perfumes For asthma, catarrh, external wounds, rheumatism
Myroxylonn balsamum (Tolu balsam)		For common cold, scabies, ulcers, wounds
Myrtus communis		Beverage
Nardophyllum lanatus	15,16-Epoxy-3β,4α-dihydroxy-7-oxo-13(16), 14-clerodadien-20,12-olide, *ent*-15,16-epoxy-3,4-seco-4(18),13(16),14-halimatriene-3,5β:20,13β-diolide	
Nasutitermes gracilirostris	1(15),8(19)-Trinervita-diene-2β,3α,9α,11-tetraacetate	
Nepeta obtusicreana	14α-Acetoxy-6-oxoabieta-7-ene, euroabienol, obtusicrenone (**153**)	Anti-Alzheimer, anticholinesterase activity
Nepeta sauvis	Nepetolide	Antiasthmatic, antispasmodic, diaphoretic, diuretic, sedative, tonic, vulnerary For abdominal spasm, calcium antagonist
Nepeta septemcrenata	1α,14α,18-Trihydroxy-7α-acetoxy-8,15-isopimara-diene, 1α-hydroxy-7α,14α, 18-triacetoxy-8,15-iso-pimaradiene	Antimicrobial
Nepeta sorgerae	14α-Acetoxy-18-hydroxy-iso-pimara-8,15-dien-7-one, sorgerolone	Acetylcholinesterase and butyrylcholine esterase inhibitory, antioxidant
Nepeta teydea	8,11,13-Abietatriene-1α, 18-diol (=teideadiol), netidial, netidiol A 7α-monoacetate, netidiol A 18-monoacetate, netidiol B, netidiol B 14α-monoacetate, netidiol B 18-acetate, netiol, 13α-isopropyl-8(14)-podocarpen-14α,18-diol, 13α-isopropyl-7(8)-podocarpen-14α,18-diol	Aphrodisiac, diuretic, hyperglycemic For catarrhal

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Nepeta tuberosa subsp. *beticulata* (cat mint)	Isopimaryl 2β[(1'-methyl-2'-al)ethyl]-5α-methyl-cyclopenta-β-carboxylate, isopimaryl 2β-acetyl-5α-methyl-cyclopentane-β-carboxylate, 3α-isopimaryloxy-4α,7α-dihydronepetalactone, isopimaryl-4β[(3'α-methyl-2'β-methoxy-carbonyl) cyclopentyl]-2-pentanoate, isopimaryl 3β -methylencarboxylate-4α,7α-dihydro-nepetalactone, iso-pimaryl-2α-methylencarboxylate-4α,7α-dihydro-nepetalactone, iso-pimaryl 3-methylencarboxylate-4-hydroxy-4α,7α-dihydronepetalactone, di-isopimaryl malonate, isopimaryl malonic acid, 7-oxo-isopymara-8,15-dien-18-ol, iso-pimaryl acetate, iso-pimarol, isopimaric acid, 8(14),15-iso-pimaradien-7α,18-diol, myrceocomunic acid, 8,11,13-abietatrien-3β-ol, iso-pimara-8,15-dien-7β,18-diol, 7α-hydroxy-iso-pimara-8(14),15-dienyl malonate, acetyl isocupressic acid	
Nidorella agria	19-Hydroxy-3,13-cloredadien-16,15-olide	
Nidorella auriculata	15,16-Epoxy-1,3,13(16), 14-clerodatetaraen-18-oic acid methyl ester (=methyl nidoresedate)	
Nigella sativa	Nigellamines A_1-A_5, B_1, B_2, B_3, C, D	Cytotoxic (HepG2: hepatoblastoma cancer cell line) For asthma, abdominal pain, flatulence, kidney stones, polio
Nothopanax simplex	α-Camphorene	
Oidiodendron truncatum	8β*H*,12*R*,13*R*,14*R*-13,16-Epoxy-3-cleroden-12,14, 15,18-tetrol	

Olea europaea var. *maderensis*	Geranyl geraniol, phytol	Beverage (herbal tea), Infusion: antihypertensive
Oospore virescens	Virescenol A, 7,15-Isopimaradiene-3,19-diol (=virescenol B), 7,15-isopimaradiene-3-hydroxy-12-*O*-β-*D*-altropyranoside, virescenoside F, G, 19-hydroxy-7,15-isopimaradien-3-one (virenosol C), virescenoside C	
Oryza sativa	Momilactone A (**154**), B (**155**), C (**156**), D, E, 6,19β-epoxy-3β-hydroxy-5α,9β-pimara-7,15-diene, (9β*H*)-pimara-7,15-diene-3β,6β-19-triol, ineketone (**157**), gibberellin A_1, A_4, A_9, A_{19}, A_{20}, A24, A_{29}, A_{34}, A_{44}, A_{51} and A_{53}, *ent*-copalyl diphosphate, *ent*-sandaracopimaradiene, oryzalexin S, stemar-13-ene, *syn*-copalyl diphosphate, oryzalexin A-F and S, *ent*-kaur-16-ene, *ent*-cassa-12,15-diene, phytocassane A-E, oryzadione	Food (rice), brown and germinated brown rice Anticancer, antidiabetes mellitus, antidiarrheal, antifungal, antimicrobial, antioxidant, antitumor, antiviral, cytotoxic, germination inhibitory For cardiovascular
Osimum labiatum (pink sage)	Labda-8(17),12*E*,14-tetrene-2*R*,18-diol	Anticancer (breast cancer), anti-inflammatory, antimicrobial, antituberculosis, cytotoxic
Osmunda japonica	*ent*-Kauran-16*S*,17-hydroxy-19-oic acid (**37**)	Food Cytotoxic (HeLa, HePG2)
Pachdictyon coriaceum	6-Acetoxydilophol, 3β-hydroxy-6β-acetoxy-dilophol	
Pachydictyon coriaceum oil	Pachydictyol	
Palafoxia arida	*ent*-7-Pimarane-2α,15,16,18-tetrol, *ent*-7-pimarane-2α,15,16,18-tetrol, *ent*-7-pimarane-2α,15,18-trihydroxy-16-*O*-β-*D*-glucopyranoside, *ent*-3α,15*S*-hydroxy-pararosane, *ent*-3β,15*S*-hydroxypararosane, *ent*-7-pimarane-2α,15,18-trihysroxy-16-*O*-β-*D*-glucopyranoside, darutigenol	
Palafoxia rosea	*ent*-7-Pimarene-3α,15*S*,16-triol, 3-hydroxypalarosane	
Palafoxia texana	jesromotetrol, 3β-acetoxy-jesromotetrol, 3β,19-diacetoxy-jesromotetrol	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Peltodon longipes	Abietanes	Cytotoxic (MIAPaCa-2: human pancreatic, MV-3: melanoma tumor cell lines)
Perovskia scrophularifolia	Rosmanol (**17**), epi-rosmanol, carnasol, 12-*O*-methyl carnosic acid	For dermatitis, intestinal parasites
Petalostigma pubescens	Pimaranes, sonderianol	Cytotoxic (L1210, P388 cell lines)
Phaieria nisidai	Simplexin	Immune protective
Phlomidoschema parviflorum	1(10),15-Rosandien-18-oic acid (**158**), 1(10),3,15-rosatrien-18-oic acid methyl ester	Muscle relaxant, sedative For cancerous ulcers, diarrhea, fever, heart genital tumors, inflammatory, internal bleeding, liver disorders, sclerosis of the spleen, sore mouth and throat, tumors
Phlomis betonicoides	Phlomoidesides A, phlomisosides III, V, baiyunol (=15,16-epoxy-8,13(16),14-labdatrien-3-ol, baiyunoside (=baiyunol-3-*O*-[β-*D*-glucopyranosyl-(1-2)-β-*D*-xylopyranoside	Cytotoxic (NCl-H, 1975, HepG2, MCF-7: human tumor cell lines)
Phlomis lychnitis	Phlomisosides II-IV (2-*O*-(6-desoxy-α-mannopyranosyl)-β-glucopyranoside)	Beverage (herbal tea) Anti-inflammatory, antioxidant, antipyretic, cold, digestive, gastrointestinal disorders, sedative, throat pain
Phlomis medicinalis	Baiyunoside, phlomisoside II, III.	Beverage (herbal tea) Antipyretic, sedative, For cold, digestive, gastrointestinal disorders, throat pain
Phlomis tuberosa	14-Hydroxyabieta-8,11,13-triene-11-carbaldehyde-18-oic-12-caboxy-13(1-hydroxymethylethyl)-lactone, 14-hydroxyabieta-8,11,13-triene-17-oic-12-caboxy-13-(1-hydroxy-1-methyl ethyl)lactone, 14,16-dihydroxyabieta-8,11,13-triene-15,17-dioic acid, 15,16-epoxy-8,13(16),14-labdatrien-19-oic acid (=phlomisol), phlomisoic acid (=15,16-epoxy-8,13(16),14-labda-trien-19-oic acid), 15,16-epoxy-8,13(16),14-labdatriene	Antimicrobial, α-Glucosidase inhibitory, intoxication, rheumatoid arthritis For cardiovascular and pulmonary diseases, tuberculosis

Phlomis younghusbandi	Phlomisoic acid phlomisosides III (=phlomisoic acid β-*D*-xylopyransyl-(1→2)-β-*D*-glucopyranoside), IV (=phlomisoic acid α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranoside)	Beverage (herbal tea)
Physcomitrella patens	*ent*-Beyerene, 16-hydroxy-*ent*-kaurene *ent*-kaur-16-ene, *ent*-sandaracopimaradiene	Antimutagenic
Picea abies	8,11,13-Abietatriene-15-hydroxyl-18-oic acid methyl ester	
Picea jezoensis	7,13-Abietadien-18-oic acid (=abietic acid) (**2**), 8(14),12-abietadien-18-oic acid (=levopimaric acid)	
Pieris japonica	2β,3β-Epoxy-5β,6β,7α,9β,10α,14β,16α-grayanotoxaneheptol	
Pinus banksina	18-Nor-8,11,13-abietatrien-4α-ol, 18-nor-8,11,13-abietatrien-4β-ol	
Pinus caribaea	8,13-Abietadien-18-oic acid	
Pinus contorta	(13*S*)-Labda-8(17),14-diene-13β,18-diol, 18-norlabda-8(17),13-diene-4α,15-diol, 8(14),15-pimaradien-18-al, 7,15-isopimaradien-18-al, 8(14),15-pimaradien-18-ol, 7,15-isopimaradien-18-ol	Edible (inner bark, sap, seed, gum) Antiseptic, diuretic For colds, coughs consumption, gonorrhea, rheumatic affections, rubefacient, stomach pains, vermifuge, vulnerary, wound healing
Pinus koraiensis	Dehydroabitane, dehydroabitanal, dehydroabietic acid methyl ester, pimara-7,15-dien-3-one	Edible (seed)
Pinus lambertiana	15,16-Epoxy-8(17),13(16),14-labdatrien-19-oic acid (=lanbertianic acid), 12-oxolanbertianic acid	
Pinus monicola	8,11,13-Abietatrien-7-ol, 8,11,13-abietatrien-7-one, 9,10β-epoxy-6,12-dihydroxy-6,7-seco-8,11,13-abietatriene	
Pinus morrisonicola (=Pinus formosana)	15-acetoxy-7-oxo-dehydroabietic acid, picealactones A-C, 7-oxodehydroabietic acid	Antihypertension, antioxidant, lipid-lowering
Pinus pallasiana	8,13-Abietadien-18-oic acid, dehydroabitane	
Pinus palustris	8,13-Abietadien-18-oic acid (=palustric acid)	Flavor Antiseptic, bactericides, disinfectant, diuretic, vermifuge For chronic, cold, colic, cough, diarrhea, worms

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Pinus pimaster	Abietatriene, abieta-7,13-diene, dehydroabietal, iso-abienol, labda-8(17)-13*E*-dien-19-al, labda-8(*17*),13*E*-dien-15-ol, labda-8(17),13*Z*-dien-15-al, neoabietadiene	Lipid for food, beverage (herbal tea) Anti-inflammatory, antimicrobial, antioxidant, antiviral, cholesterol lowering effect, endometriosis For allergy, asthma, cardiovascular, menstrual and venous disorders, pregnancy associated pain
Pinus pityusa	8,13-Abietadien-18-oic acid	
Pinus pumila	*ent*-12,15-Epoxy-8(17),12,14-labdatrien-7α-ol (=pulmiloxide) *ent*-7β-hydroxypumiloxide	
Pinus radiata	13β-Hydroxy-18-abietanoic acid	
Pinus strobus	12*E*,14-Labdadien-8α-ol (=abienol), strobal	
Pinus sylvestris	8α,13*R*-Epoxy-14-labden-19-oic acid, (= dehydropinifolic acid), 8(17)-labdadiene-15,18-dioic acid, 13(16),14-labdadien-8α-ol (=iso-abienol), 13*S*,8(17)-labdane-15,18-dioic acid (=pinifolic acid), 7,13-abietadien-18-ol (=abietinol), 7,13-abietadien-18-al (=abietinal), 8,11,13-abietatrien-19-acetate (=dehydroabietinol acetate), 8α-hydroxy-12-oxo-13-abieten-18-oic acid, 7,15-isopimaradiene (=isopimaradiene), 19-nor-8(14),15-iso-pimara-dien-3-one, 15-ethyl-18-methyl pinifolate, 18-hydroxylabda-8(17), 13*E*-dien-15-acetate	Edible (pine nut) Cytotoxic (BEL-7402, HeLa, SK-N-SH cancer cell lines), improves vision, maintains weight stability, lowers bad cholesterol, and benefits bone strength, reduces the risk of heart attack and boost energy levels
Pisum bulgaris	Gibberellin A_{44}	
Pisum sativam	Gibberellin A_{44}	
Piteris livida	*ent*-9α-Hydroxy-15-oxo-16-kauren-19-oic acid β-*D*-glucopyranosyl ester, *ent*-6α,9α-dihydroxy-15-oxo-16-kauren-19-oic acid β-*D*-glucopyranosyl ester, *ent*-6α,11α-dihydroxy-15-oxo-16-kauren-19-oic acid β-*D*-glucopyranosyl ester	

Piteris longipes	*ent*-11α,15α-Epoxykaura-19-oic acid, *ent*-9α,15α-dihydroxy-16-karen-19-oic acid, *ent*-11α,15α-hydroxy-15-oxo-19-kauren-19-oic acid, *ent*-11α,15α-hydroxy-15-oxo-19-kauren-19-oic acid β-*D*-glucopyranosyl ester, *ent*-11α-hydroxy-15-oxo-19-kauranoic acid	
Pittisporum undulatum	8β,13β-Dihydorxykaur-16-ene	
Pityrodia lepidota	6α,18-Dihydroxy-2,13*E*-clerodadien-15-oic acid methyl ester	
Plantago lanceolata	Phytol, phytone	Beverage (herbal tea) Antidote, Anti-inflammatory, antimicrobial, antioxidant, antispasmodic, antitumor, cytotoxic, respiratory tract disorders For conjunctivitis, furunculosis, decoction or juice: eye drops for conjunctivitis, poultices for cleansing open sores
Plathymenia reticulata	*ent*-5α-3,14-Clerodadien-13-ol (= platyterpol)	
Platycaete aucheri	*ent*-7-Oxo-3,13*E*-clerodadien-15-oic acid, 6β-hydroxy-7-oxo-3,13-clerodadiene-15-oic acid, *ent*-6β-hydroxy-7-oxo-3,13-clerodadien-15,16-olide	
Plectranthus barbatus (=*Coleus barbatus, Coleus forskohlii*)	7,20-Epoxy-8,11,13-abietatriene-11,12-diol (=20-deoxocarnosol), cariocal, 6,11-epoxy-6α,12-dihydroxy-6,7-seco-8,11, 13-abietatrien-7-al, 19(4β→3β)abeo-6,11-epoxy-6α,12-dihydroxy-6,7-seco-4(18),8,11,13-abietatetraen-7-al, barbatusol, coleon E, F, 9(10→20)abeo-8,11,13-abietatriene-10β,11,12-triol, 6,11-epoxy-6α,12-dihydroxy-6,7-seco-4 (18),8,11,13-abietatetraen-7-al, cyclobutatusin, cyclobarbatusin	Beverage (herbal tea) Acetylcholinesterase inhibitory, antiprotozoal
Plectranthus ernstii	*rel*-15ξ,16-Epoxy-7α-hydroxypimar-8,14-ene, *rel*-15ξ,16-epoxy-7-oxopimar-8,14-ene, 1*R*,11*S*-dihydroxy-8*R*,13*R*-epoxylabd-14-ene	Antibacterial

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Plectranthus fruticosus	*ent*-Labda-8(17),12*Z*-trien-2α-ol, *ent*-2α-acetoxylabda-8 (17),12*Z*,14-trien-3β-ol, 3β-acetoxylabda-8(17), 12*E*,14-trien-3α-ol, *ent*-15β,16β-epoxykauran-19-oic acid, *ent*-15β,16β-epoxykauran-19-ol	Antimicrobial
Plectranthus nummularis	Parvifloron E, parvifloron F, 19-*O*-3,4-dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-abeoabieta-1(10),6,8,11, 13-tetraene, 12-*O*-(3-methyl-2-butenoyl)-19-*O*-(3,4-dihydroxybenzoyl)-11-hydroxyabieta-8,11,13-triene	
Plectranthus barbatus	Prectrin, 15*S*,6β,12α-acetoxy-7α-hydroxy-13β,16-cycloabieta-8-ene-3,11,14-trione (=barbatusin), 15*S*,19(4→3)-abeo-6β,7α,12α-trihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione (=7,12-bis(*O*)-desacetyl) coleon N, 19(4→3),17 (15→16)bis-abeoabieta-11,12,14,16-tetrahydroxy-3,5,8,11,13-pentene-2,7-dione (=plectrione A)	Anti-Alzheimer disease, antifeedant, antifungal, anti-inflammatory, antileishmanial, antimicrobial, antioxidant For dental caries, digestive, fever, genitourinary, heart blood and circulatory, infection, musculoskeletal disorder, pain, poisons treatment, respiratory, sensory
Plectranthus caninus	3β,6,11,12,14-Pentahydroxy-5,8,11,13-abietatetraen-7-one (= coleon S), coleon T, 7,12-diacetylcoleon J, coleon Q (**71**), coleon R	For digestive, respiratory conditions
Plectranthus coesta	3α,7β,14α,20-Tetrahydroxy-16-kauren-15-one (=coestinol)	
Plectranthus ecklonii	Plavifloron D	Antioxidant, cytotoxic (breast, colon, glioma, leukemia, lung, melanoma, pancreatic, cancer cell lines, P-glycoprotein-over expressing cells) For skin condition
Plectranthus edulis	Edulone A-11,12-diacetate , 6β,7α-dihydroxyroyleanone, lanugone A, 3α-formyloxy-6β,7α,12-trihydroxy-17(15→16) abeoabieta-8,12,16-trinene-11,14-dione, 3α-formyloxy-12-hydroxy-17(15→16)abeo-abieta-6,8,12,16-tetraene-11,14-dione,17(15→16), 19α(4→3) bisabeo-6β,7α,12,16abietatetraene-11,14-dione	For digestive, respiratory conditions

Plectranthus elegans	11-Hydroxy-7,9(11),13-abietatriene-12-one, 7α,11-dihydroxy-12-methoxy-8,11,13-abietatriene	Antimicrobial For genitourinary condition
Plectranthus ernstii		Antimicrobial
Plectranthus grandidentatus	11,14-Dihydroxy-7,9(11), 13-abietatriene-6,12-dione, grandidone A, B, 7-*epi*-grandidone A-D, 12-Hydroxy-8,12-abietadiene-11,14-dione (=royleanone), 7α,12-dihydroxy-8,12-abietadine-11,14-dione (=horminone), 6β-hydroxyroyleanone, 6β,7α-dihydroxyroyleaonone, 7α-acetoxy-6β-hydroxyroyleanone, 6,7-dehydroroyleanone, 6,11,12,14-tetrahydroxy-5,8,11,13-abietatriene-7-one (=coleon U)	Antimicrobial (methicillin and vancomycin resistant), antiproliferative (human lymphocytes), antitumor (breast, lung, renal melanoma, CNS) For blood and heart circular, respiratory condition
Plectranthus hadiensis	6β,7α-Dihydroxyroyleaonone, 7α-acetoxyroyleanone, 7α-formyloxy-6β-hydroxy-royleanone	Antifungal, anti-inflammatory, antimicrobial, antioxidant For digestive, respiratory conditions, skin disorders
Plectranthus hereroensis	7α,12-Dihydroxy-8,12-abietadine-11,14-dione (=horminone), 16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione	For digestive condition
Plectranthus incanus (=*P. mollis*)		Vegetable Anticancer, antidote, astringent, anti-inflammatory, antioxidant, antirheumatism, antitumor, cardiac depressant, cytotoxic, febrifuge, muscle relaxant, mosquitocidal, mosquito repellent, respiratory stimulant, tonic For bleeding stop, fever, snake bites, vasoconstriction
Plectranthus lanuginosus	Lanugone A-S, K', K"	Anti-Alzheimer disease, antioxidant For digestive condition
Plectranthus madagascariensis	7α-Acetoxy-6β-hydroxyroyleanone, 6,7-dehydroroyleanone, 7α-fromyoxy-6β-hydroxy-royleanone, 7α-acetoxy-6β-hydroxy-royleanone, 6,11,12,14-Tetrahydroxy-5,8,11,13-abitatetraen-7-one (=coleon U), 7β,6β-dihydroxyroyleanone, parvifloron D	Cytotoxic (CCRF-CEM: human leukemia, A549: lung adenocarcinoma cell line) For asthma, chest complaints, colds, coughs, enema

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Plectranthus myrianthus	Coleon U, 7α-formoxy-6β,12,16-trihydroxy-8,11,13-abietatrien, 11,12,14-trihydroxy-8,11,13-abietatriene-6,7-dione (=coleon V), epi-grandidone A, grandidone B, C, D, 7-epi-grandidone B, 3-epi-grandidone	
Plectranthus nilgherricus	11,15-Dihydroxy-5,7,9(11),13-abietatetraene-12-one (=fuerstione)	
Plectranthus parviflorus	11-Hydroxy-19-(3-methyl-2-butenoyl)-5,7,9(11),13-abietateraen-12-one (=paviflorone A) parviflorone D, 11-hydroxyl-19-(3,4-dihydroxybenzoyl)-5,7,9(11),13-abietateraen-12-one (=parviflorone E), 2-(3,4-dihydroxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraen-12-one (=paviflorone F)	For fever, infection
Plectranthus puberulentus	4*R*,19*S*-7,12,19α-Trihydroxy-1,5(10),6,8,12-abietapentene-11,14-dione (=coleon A), 7,12-dihydroxy-1,5(10),6,8,12-abietapentaene-11,14,19-trione (=coleon A lactone)	Antifeedant, antimicrobial
Plectranthus scutellaioides	Spiroscutelone A (**159**), B, C	Cytotoxic (Hela, MCF-7, PSN-1) For abdominal pain, emmenagogue, skin disorders
Plectranthus sanguineus	7α,12-Dihydroxy-8,12-abietadiene-11,14-dione (=horminone), 7α-formyloxy-12-hydroxy-8,12-abietadiene-11,14-dione (=7-O-formylhorminone), sanguinone A, 6β-hydroxyroyleaonone (=6β-hydroxyroyleanone), 7α-acetoxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione (=7α-acetoxy-6β-hydroxyroyleanone), 7α-formyloxy-6β-hydroxyroyleanone, 5,6-dihydrocoleon U, coleon U quinone, 8α,9α-epoxycoleon U quinone, grandidone A, B, 7-*epi*-grandidone A, B	
Podocarpus cupressina	12-Hydroxy-8,11,13-podocarpatriene-19-oic acid	

Podocarpus dacrydioides	5β*H*,12-hydroxy-8,11,13-abietatriene-6,7-dione, 12,16,17-trihydroxy-8,11,12-abietartrien-19-oic acid (=pododacric acid)	
Podocarpus ferrugieus	Kaur-16-ene	
Podocarpus ferrugineus	8,11,13-Abietatriene, 12-hydroxy-8,11,13-abietatriene-2-one (=2-oxoferruginol), 8,11,15-abietatrien-12-ol (=ferruginol), 8,11,13-abietatriene-12,19-diol, 12-hydroxy-8,12-abietadiene-11,14-dione (=royleanone), cupresol, cupresol methyl ether, kaur-16-ene	
Podocarpus gracilior	Podlide	
Podocarpus hallii	Hallactone A, podolactone C S-oxide, 8,14-isopimarene-2,15,16,18-tetrol (=hallol)	
Podocarpus lambertius	8,11,13-Abietatrien-19-oic acid (=lambertic acid), macrophyllic acid	
Podocarpus macrophylla	Macrophyllic acid, podototarin	
Podocarpus macrophyllus (Japanese yew)	Podocarpone E 7α,8α-epoxide (=inumakilactone B), inumakilactone A, E, ponalactone A, E	Edible (Fruits) Fruit: carminative. pectoral and stomachic, seeds: cholera, heat ailments, stomach diseases, for sweet Leaves: (decocted) For rheumatism, joint painful Fruits (decocted) Antiseptic, astringent, carminative, tonic For arsenic poisoning, fever, heart, kidney, lung, stomach, skin and ulcer diseases
Podocarpus mannii (*Afrocarpus mannii*)	Podototarin, 8,11,13-totaratriene-13,19-diol (=hydroxytotarol), 13-hydroxy-8,11,13-totaratrien-19-al, 13-hydroxy-8,11,13-totaratrien-19-oic acid	
Podocarpus milanjiamus	Milanjilactones A, B, milanjilactone B 7α,8α-epoxide	
Podocarpus nagi	Nagilactones A (**14**), B-D, podototarin, 15-methoxycarbonylnagilactone D	Antitumoral
Podocarpus nakaii	Ponalactone A, ponalactone A β-D-glucopyranoside	
Podocarpus neriifolius	Podolactone C, D	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Podocarpus nubigena	Nubilactone A	
Podocarpus polystachyus	Podocarpone E 7α,8α-epoxide (=inumakilactone B)	
Podocarpus saligna	Salignone A, B, D, I-L	
Podocarpus sellowii	Sellowin A, B, 2β,3β-epoxysalignone H	
Podocarpus spicatus	16-Kaurene	
Podocarpus totara	Podocarpic acid, totarol (**180**), 8,11,13-abietatrien-12-ol (=ferruginol), podototarin, 8,11,13-totaratriene-13,19-diol (=hydroxytotarol), 13-hydroxy-8,11,13-totaratrien-19-oic acid, 8,11,13-podocarpatriene-12,19-diol (=podocarpic acid)	Anti-influenza, *anti*-microbial
Podocarpus urbanii	Urbalactone	
Polyalthia longifolia	16*R*-Hydroxy-3,13-cloerodadien-15,16-olide, 16-oxo-3,13*E*-clerodadien-15-oic acid, 16-hydroxycleroda-3,15-dien-16,15-olide, 16-oxocleroda-3,13*E*-dien-15-oic acid, 3,16-dihydroxy-cleroda-4(18),13,14*Z*-dien-15,16-olide,16α-hydroxy-cleroda-3,13,14*Z*-dien-15,16-olide	Antiadipogenic, antimicrobial, antiobesity, anti-*Plasmodium falciparum*
Polyalthia viridis	*ent*-16-Hydroxy-2-oxo-3, 13-clerodadien-15,16-olide (=2-oxo-3,13-kolavadien-15,16-olide), (4→2)-abeo-16-hydroxy-3-oxo-clerodadien-15,16-olide (= (4→2)-abeo-3-oxo-2,13-kolavadien-15,16-olide	
Polygonum aviculare	Diterpene alkaloid: 6-hydroxy-11-dexoy-1,3-dehydrohetisane (= panicudine) (**160**)	Beverage (herbal tea) For antirheumatism Infusion: diuretic, antimicrobial

Portulaca cv. jewel	3,13*Z*-Clerodadiene-15,16,18,19-tetrol (=jewnol A), *ent*-15,16-dihydroxy-17-oxo-3,13-clerodadien-18,19-olide (=portulide D), *ent*-7,15,16,19-tetrahydroxy-3,13-clerodadien-18-oic acid (=portulide C), portulide B, *ent*-15,16-dihydroxy-3,13*Z*-clerodadien-18,19-olide	
Portulaca grandiflora	*ent*-15,16,17-Trihydroxy-3,13Z-clerodadien-1,18-olide (=portulide, 3-hydroxyportulol ether, portulal	Vegetable (used in salads, cooked like spinach) For burns, cirrhosis of the liver, eczema, hepatitis, insect bites, snake bite, scalds, swelling and pain in the pharynx
Portulaca oleracea	Potulene	Antifungal, antimicrobial, hepatoprotective
Premna herbacea	Sirutekkone	Edible (fruits), Beverage (juice from roots and rhizomes) For dropsy, cough, asthma, fever, cholera, and rheumatism
Premna integrifilia	11,12,16-Trihydroxy-5,8,11,13-abietatetraen-7-one, 12,16-epoxy-11,14-dihydroxy-5,8,11,13-abietatetraen-7-one, 12,16-epoxy-11,14-dihydroxy-5,8,11,13-abietatetrane-7-one	
Premna latifolia	6α,11,12,16-Tetrahydroxy -8,11,13-abietatrien-7-one (=nellionol), 11,12,16-trihydroxy-5,8,11,13-abietatetraen-7-one	Cytotoxic (A-431, A-549, ACHN, B-16, F10, Hep-G2, HT-29, MCF-7, PC-2 cancer cell lines)
	Icetexanes, premnalatifolin A	Cytotoxic (MCF-7: breast, HT-29 cancer cell lines
Premna obtusifoloa	Acanthoic acid analogues, pimaranes, rosanes, abietanes, icetexanes, rearranged icetexanes,	Antimicrobial, NO production inhibitory
Premna schimperi	5*R*,8*R*,9*S*,10*R*-12-Oxo-*ent*-3,13(16)-clerod-15-oic acid, 16-hydroxy-clerod-3,13(14)-dien-15,16-olide, *ent*-12-oxolabda-8,13(16)-dien-15-oic acid, andrographolide, *ent*-12-oxo-3,13 (16)-clerodadien-15-oic acid	Antibacterial, antileishmanial

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Premna tomentosa	Icetexanes	Antioxidant, α-glucosidase inhibitory
Printzia laxa	Printzianic acid, isoprintzianic acid	
Prioria balsamifera (=Gossweilerodendron balsamiferum)	*ent*-15,16-Epoxy-4(18),13(16),14-cleroda-trien-3β-ol (=agbaniol)	
Propolis	Manoyl oxide, labda-8(17),12,13-triene, 13-*epi*-manool, communal, sempervirol, ferruginol, 14,15-dinor-13-oxo-8 (17)-labden-19-oic acid, copalol, 18-hydroxyabieta-8,11,13-triene, communic acid, totarol, 13-*epi*-torulosal, neoabietic acid, pimaric acid, imbricatoloic acid, 13-*epi*-torulosol, abietic acid (**2**), dehydroabietic acid (**43**), 13-*epi*-cupressic acid, dihydroxyabieta-8,11,13-triene, isoagatholal, 2-hydroxyferruginol, hydroxydehydroabietic acid, agathadiol, totarolon, isocupressic acid, 6,7-dihydroxyferruginol, ferruginolon, junicedric acid, acetylisocupressic acid, 13(14)-dehydrojunicedric acid, 13-succinyloxy-abietadiene, 13-succinyloxyabieta-8,11,13-triene, 13-succinyloxyabietadiene isomer, 13-succinyloxyhydroxy abietadiene	Foods Antibacterial, anti-inflammatory, antimycotic, antioxidant, antiviral, cytotoxic, immunomodulating For burns, wound healing
Prumnopitys andina	2β-Acetoxyferrugiol	Antimicrobial
Prunella vulgaris	Vulgarisins A-D	Antibacterial, anticancer, antidiabetes, anti-inflammatory, antiviral (for Helpes), cytotoxic (A549: human lung carcinoma cell line) For wound healing
Pseudopterogorgia bipinnata	Bipinnapterolide	Antimicrobial

Psidium cattleynum (=*P. cattleinum*)	3β-Kauren-18-yl acetate, phytol, thunbergol	Food, syrup, ice cream, jams, jellies, beverage (herbal tea) Infusion: antidiabetes, antineoplastic, antioxidant, astringent
Pteris cretica	2β,15α,19-Trihydroxy-*ent*-kaur-16-ene 2-*O*-β-glucopyranoside, 2β,6β,15α-trihydroxy-*ent*-kaur-16-ene 2-*O*-β-*D*-allopyranoside, 2β,6β,16α-trihydroxy-*ent*-kaurane 2-*O*-β-*D*-allopyranoside, 2β,16α,19-trihydroxy-*ent*-kaurane 2-*O*-β-allopyranoside	
Pteris dipar	5β,11β,12β-Trihydroxy-15-oxo-*ent*-kaur-16-en-19-oic acid (**38**), 7β,9-dihydroxy-15-oxo-*ent*-kaur-16-en-19,6β-olide, (16*R*),11β-hydroxy-15-oxo-*ent*-kaur-19-oic acid, (16*R*)-11β-hydroxy-15-oxo-*ent*-kaur-19-oic acid 19-β-*D*-glucoside	Cytotoxicity (KB cell)
Pteris livida	*ent*-9α-Hydroxy-15-oxo-16-kauren-19-oic acid β-*D*-glucopyranosyl ester, *ent*-6α,11α-dihydroxy-15-oxo-16-kauren-19-oic acid β-*D*-glucopyranosyl ester	
Pteris longipes	*ent*-9α,15α-Dihydroxy-16-kauren-19-oic acid, *ent*-9α-hydroxy-15-oxo-16-kauren-19-oic acid (=pterokaurene L_1), *ent*-12α,15α-dihydroxy-16-kauren-19-oic acid (=pterokaurene L_4), 11α,16α-epoxy-19-kauranic acid (=pterokaurene L_5)	
Pteris multifida	*ent*-2α,6α,15β-Trihydroxykaur-15-ene (**161**), *ent*-2α,15β-dihydroxykaur-15-ene, *ent*-2α,16β-dihydroxy-kaurane, 2α-*O*-β-*D*-glucopyranosyl-15β-hydroxylkaur-15-ene	Chinese medicine Antidysenteric, anti-inflammatory, antioxidant, antipyretic, antirheumatism, detoxicant, acne, appendicitis, bleeding
Pteris plumbaea	*ent*-2α,14α,15β,16β,17-Kauranepentol	
Pteris purpureorachis	*ent*-9α-Hydroxy-15-oxo-16-atisen-19-oic acid (=pteriatisene P_1), *ent*-9α,15α-dihydroxy-16-atisen-19-oic acid (=pteriatisene P_2)	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Pteris semipinnata	*ent*-11α-Hydroxy-15-oxo-kaur-16-ene (**5**)	Chinese medicine Anticancer, apoptosis, cytotoxic (HT-29 and MNK-45 human colon cancer cells), snake bites
Pteronia divaricata	3-Hydroxypteronialacone, 3-oxopteronialacone	
Pteronia eenii	19-Hydroxypteronia dilactone (=pteroniatrilactone), 15,16-Epoxy-3α,4β,8β,10β,-tetrahydroxy-13(16),14-clerodadien-20,1-olide, 15,16-epoxy-3α-oxo-4β,8β,10β,-trihydroxy-13(16),14-clerodadien-20,1-olide, pteroneeniol, secoeeniolide	
Pteronia incana	15,16-Epoxy-3α,4β,10β-trihydroxy-13(16),14-clerodadien-20,1-olide, 15,16-epoxy-3α-acetoxy-4β,8β,10β,-trihydroxy-13(16),14-clerodadien-20,12-olide, 3α,4α:8β,12:15,16-triepoxy-10-hydroxy-13(16),14-clerodadien-20,1β-olide, incanpteroniolide	
Pteronia paniculata	7-Oxo-3-cleroden-15-oic acid (=7-oxo-13,14-dihydrokolvanic acid), 3-cleroden-15-oic acid	
Pulicaria gnaphaloides	15,16-Epoxy-3,13(16),14-clerodatrien-18,6-olide	Antioxidant
Pulicaria salviifolia	*ent*-15,16-Epoxy-6β,7α-dihydroxy-3,13(16),14-clerodatrien-18-oic acid, *ent*-15,16-epoxy-3,13(16),14-clerodatrien-18,6-olide (=salvin)	Antioxidant
Pycnocycia spinosa	3,7,10,15-Pentaacetyl-5-butanoyl-13,17-epoxy-8-myrsinene	Antispasmodic
Pygmaecopremna herbacea	3β,11-Dihydroxy-5,7,9(11),13-abietateraene-2,12-one (=isobharangin), bharanginin, pimaeocin A, pytmaoecin C	
Pyllostachys edulis (bamboo shoot)	Gibberellin A_{21}	

Rabdosia adenantha	*ent*-1β,7α,11α-Triacetoxy-3α-hydroxy-16-kaurene-6,15-dione (=adenanthin)	Anti-inflammatory, bacteriostatic
Rabdosia amethystoides	ent-7β,14α,20-Trihydroxy-16-kaurene-11,15-dione (=amethystoidin A)	Chinese medicine For treating acute lymphocyte leukemia, cervical cancer
Rabdosia coetsoides	Plecostonol, *ent*-3α,7β,14α),20-tetrahydroxy-16-kauren-15-one (=coestinol)	Beverage (herbal tea), cooked with meat (Potherb)
Rabdosia effusus (=*Isodon effusus*)	*ent*-7,20-Epoxy-1β,6α,7-trihydroxy-16-kauren-15-one (= effusanin A), *ent*-7,20-epoxy-1β-acetoxy-6α,7-dihydroxy-16-kauren-15-one (= effusanin B), effusanin C, D	Antibacterial
Rabdosia eriocalyx (=*Isodon eriocalyx*)	*ent*-15α-Acetoxy-3,20-epoxy-6α-hydroxy-16-kaurene-1,7-done (=maoecrystal A), *ent*-7,20-epoxy-16-kaurene-1α,3α,6α,15α-tetrahydroxy-19-*O*-β-D-glucopyranoside (=1α,3α,6α,15α-rabdoside 2), *ent*-19-acetoxy-7,20-epoxy-1α,3α,6α,7-tetrahydroxy-16-kauren-15-one (= maoecrystal I), neolaxiflorin A, B, eriocalyxins C−E, laxiflorins J−M, maoecrystal P, maoeriocalysins A-D	Cytotoxic (K562, T24 human tumor cells)
Rabdosia excisa	Excisanin H-K, kamebakaurin *ent*-1β,7β,12β,14α-tetrahydroxy-16-kauren-15-one (=excisanin A), ent-1β,7β,14α-trihydroxy-12β-acetoxy-kaur-16-en-15-one (=excisanin B)	Anti-inflammatory cytotoxic (P-388: murine leukemia cell)
Rabdosia flexicaulis (=*Isodon flexicaulis*)	*ent*-20-Acetoxy-7β,11α,14α-trihydroxy-kauren-15-one, flexicaulin A, *ent*-1β,7β,14α-trihydroxy-20-acetoxy-16-kauren-15-one (=henryin A)	
Rabdosia henryi (=*Isodon henryi*)	*ent*-1β,7β,14α-Trihydroxy-20-acetoxy-16-kauren-15-one (=henryin A), *ent*-1β,7β,14α,20-tetrahydroxy-16-kauren-15-one (=kamebakaurin), *ent*-7β,14α,18-trihydroxy-16-kaurene-11,15-dione (=4-epihenryin A), exidonin, dophyllin G, lasiokaurin, epi-nodosin, isodonhenrins A-E	Chinese Folk medicine Cytotoxic (HL-60, SMMC-7721, A-549, MCF-7, SW480 human tumor cell lines)

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Rabdosia inflexa (=*Isodon inflexus*, Plectranthus *inflexus*)	*ent*-1β,3α-Diacetoxy-16-kaurene-6β,11α,15-triol (= inflexarabdonin A), inflexarabdonin B, E, 1α,3α-diacetoxy-6β,11β-dihydroxy-16-kauren-15-one (=*ent*-inflexinol), *ent*-1α,3α-diacetoxy-11β-hydroxy-16-kaurene-6,15-dione (=inflexin)	Anti-inflammatory, gastroprotective, NF κB and NO production inhibitory For inflammatory diseases
Rabdosia kunmingensis	*ent*-7β,11α,12β-Trihydroxy-14α-acetoxy-16-kauren-15-one (= rabdokunmin A) *ent*-7β,11α,12β,14α,18-pentahydroxy-16-kauren-15-one (=rabdokunmin E), rabdokunmin B-D, *ent*-7β,11α,12β,14α-tetrahydroxy-16-kauren-15-one (= rabdoloxin B)	
Rabdosia latifolia	*ent*-1β-Acetoxy-3β,7β,14α-trihydroxy-16-kauren-15-one (=reniformin B), *ent*-1β,7β,14α-trihydroxy-20-acetoxy-16-kauren-15-one (=henryin A)	Cytotoxic (A-549, HepG2, MCF-7, BCG-823, HeLa cancer cell lines)
Rabdosia liangshanica	7β,14α-Dihydroxy-1,16-kauradiene-3,15-dione (=liangshanin A), liangshinin B-F, *ent*-11α,16α-epoxy-17-kauranol	Anti-hepatitis virus
Rabdosia longituba	Effusanin B, *ent*-1β,7β,14α,20-tetrahydroxy-16-kauren-15-one (=kamebakaurin), *ent*-7,20-epoxy-6α,7,24α-trihydroxy-16-karen-15-one (=longikaurin A), longikaurin B, *ent*-6α,15α,20-triacetoxy-2,16-kauradiene-1,7-dione (=longirabdosin), isolongirabdiol, lasiokaurin, oridonin (**139**), rabdokaurin B, C (**162**), D (**163**), rabdophyllin G	Antimicrobial, antitumor
Rabdosia lophanthoides	6β,12-Dihydroxy-7α-methoxy-16-acetoxy-8,12-abietadiene-11,14-dione (=lophansthoidin A), 6β,12-dihydroxy-7α,16-diacetoxy-16-kauren-8,12-abietadiene-11,14-dione (=lophansthoidin B), lophansthoidin C-F	For hyperlipidemia

Rabdosia macrocalyx	*ent*-7,20:14,20-Diepoxy-1β-6α,11α,16-kauren-15-one (=macrocalin B), *ent*-1β,7β,12β,14α-tetrahydroxy-16-kauren-15-one, *ent*-1β,7β,12β,14α-tetrahydroxy-16-kauren-15-one (=excisanin A), *ent*-1β,7β,14α-trihydroxy-12β-acetoxy-16-kauren-15-one (=excisanin B), macrocalin A, macrocalysin	
Rabdosia macrophylla	*ent*-7β,14α,20-Trihydroxy-16-kaurene-11,15-dione (=amethystoidin A), *ent*-1β-acetoxy-7,20-epoxy-6α,7,14α-trihydroxy-16-kauren-15-one (=lasiokaurin)	Antitumor
Rabdosia nervosa	*ent*-6α,15α-Diacetoxy-7,20-epoxy-3α,7-dihydroxy-16-kauren-1-one, 3β-hydroxy-1α,7β,11β, 15β-tetraacetoxy-*ent*-kaur-16-en-6-one	For acute hepatitis, snake bites
Rabdosia parvifolia	*ent*-Isopimarene-6β,7β,8β-triol, *ent*-iso-pimar-15-en-6α,7α,8α-triol, parvifoline A, B, *ent*-3α-acetoxy-iso-pimar-15-en-8α-ol	
Rabdosia phyllostachys	Phyllostachysin A, B, 6β,11β,14β-trihydroxy-15α-acetoxy-*ent*-kaur-16-en-7-one-20-al	Antineoplastic
Rabdosia pseudo-irrorata	*ent*-3β,7β,14α-Trihydroxy-16-kauren-15-one (=pseurata A), *ent*-3β,7β,11β,14α-tetrahydroxy-16-kauren-15-one (=pseurata B), pseurata D-F	For eye inflammatory, round worm killing
Rabdosia rosthornii	*ent*-11α,19-Diacetoxy-7β,13β-dihydroxy-16-kauren-15-one (=rosthornin B), A-D, *ent*-7,20:14,20-diepoxy-1β,6α,7-trihydoxy-16-kauren-15-one (=ponicidin), *ent*-11α-acetoxy-13β,19-dihydroxy-16-kauren-15-one (=rosthorin)	Antibacterial
Rabdosia rubescens	*ent*-7,20-Epoxy-16-kaurene-6α,11α,14α,15α-tetraol (=rubescensin C), *ent*-2α,3α,6α,7α,11β-tetraacetoxy-7-hydroxy-16-kauren-15-one (=lushanrubescensin), lushanrubescensin B-E, *ent*-7,20:14,20-diepoxy-1β,6α,7-trihydoxy-16-kauren-15-one (=ponicidin), 3α,6β,11β,12β,-tetrahydroxy-16-kauren-15-one (=xindongnin B)	For prostate cancer, oral infection, enlarged prostate, prostatic hyperplasia

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Rabdosia serra	*ent*-7,20:18,20-Diepoxy-1β,14-dhydroxy-16-kauren-15-one (=rabdoserrin A), *ent*-1β,7β,12β,14α-tetrahydroxy-16-kauren-15-one (=excisanin A), *ent*-1β,7β,14α-trihydroxy-12β-acetoxy-16-kauren-15-one (=excisanin B), *ent*-1β,7β,14α,20-tetrahydroxy-16-kauren-15-one (=kamebakaurin), *ent*-1α,7β,14α-trihydroxy-12β-acetoxy-16-kauren-15-one (=rabdoserrin D)	Antimicrobial, anticancer (HepG2), anti-hepatitis B virus (anti-HBV)
Rabdosia shikokiana	Shikoccin (**164**), shikoccidin (**165**), *ent*-7,20-Epoxy-16-kaurene-6α,7α,11β,15α-tetrahydroxy-1-*O*-β-*D*-glucopyranoside (=shikokiaside A), rabdosichuanin D, rabdosianin A, B, shikokianoic acid, 11-acetoxyshikokianoic acid-6-al	Cytotoxic (anti-Ehrlich carcinoma)
Rabdosia ternifolia	*ent*-7,20-Epoxy-1β,6α,7,11α-tetrahydroxy-16-kauren-15-one (=lasiodonin), rabdoternin A, ternifolin, isodonoic acid, isodonal, longikaurins A, E	
Rabdosia trichocarpa	Tricholabdal A, H, 3-deoxytrichorabdol D (=trichorabdal B), trichorabdal D, 11-deoxytrichorabdal D (=tricholabdal C)	Deodorizing effect Antibacterial, antimutagenic, antitumoral, bitter stomachic, deodorizing
Rabdosia weissiensis	*ent*-1β,3α,6β,7α,11α-Pentahydroxy-16-kauren-15-one (=weissiensis A)	
Radix miltiorrhizae	Miltionins A-D	Anti-influenza A
Ramalina hierrensis (Lichen)	(-)-*ent*-kauran-16α-ol (**36**), (-)-Sandaracopimaric acid (**35**)	Anti-inflammatory, cytotoxic
Ramalina tumidula (Lichen)	(-)-*ent*-kauran-16α-ol (**36**), (-)-Sandaracopimaric acid (**35**)	Anti-inflammatory, cytotoxic

Relhania calycina	*ent*-3α,15-Dihydroxy-8,14-pimarene-16-*O*-β-*D*-glucopyranoside, 2α,16β-17-trihydroxy-3-kauranone, *ent*-2α,16β-dihydroxy-3-kauranone-17-*O*-β-*D*-glucopyranoside	
Relhania corymbosa	*ent*-Nor-3,5-clerodadien-15-ol, 19-nor-3-oxo-4-cleroden-15-oic acid	
Relhania genistifolia	3α,4α-Dihydroxy-15-clerodanoic acid, *ent*-3α,4α-dihydroxy-13-cleroden-15-oic acid, *ent*-3,7α-dihydroxy-2-oxocleroden-15-oic acid, *ent*-3α-hydroxy-7α-acetoxy-cleroden-15-oic acid methyl ester, *ent*-7α-acetoxy-5-oxo-4(19)-cleroden-15-oic acid	
Relhania squarosa	*ent*-2-Hydroxy-3-oxo-1,4(19)-clerodadien-15-oic acid, *ent*-3-oxo-4(19)-cleroden-15-oic acid	
Rhododendron japonicum	2,3-Epoxy-6β,14β-diacetoxy-5β,10α,16α-grayantoxanetriol (=rhodojaponin I), 2,3-epoxy-6-acetoxy-5β,10α,14β,16α-grayanotoxatetrol (=rhodojaponin II), 2,3-epoxy-5β,6β,10α,14β,16α-grayantoxanepentol (=rhodojaponin III), rhodojaponin IV, 2,3-epoxy-14-acetoxy-5β,6β,10α,16α-grayanotoxatetrol (=rhodojaponin V), rhodojaponin VII	Cytotoxic
Rhynchospermum verticillatum	*ent*-15,16-Epoxy-3,13(16),14-clerodatrien-18,19-olide-12*R*-*O*-β-*D*-glucopyranoside (=rhynchospermoside A), *ent*-15,16-epoxy-3,13(16),14-clerodatrien-18,19-olide-12*S*-*O*-β-D-glucopyranoside (=rhynchospermoside A), *ent*-15,16-epoxy-1-oxo-2,13(16),14-clerodatriene-17,12:18,19-diolide (=rhynchosperin A), rhynchosperin B, C	
Roylea calycina	Calyone (=3-acetoxy-15,16-epoxy-9-hydroxylabdα-13(16),14-dien-7-one), calyenone [=3-acetoxy-15,16-epoxylabdα-8,13(16)-trien-7-one], precalyone (=3-acetoxy-9,13;15,16-diepoxylabda-14-en-7-one)	Antitumor (lymphocytic leukemia)

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Rubus chingi	Labdanes	Cytotoxic (A549, A2780, BGC-823, HCT-8, MCF-7, M231, NP14, NTUB1 cancer cell lines)
Rubus orchorifolius	*ent*-Kaur-2-oxo-16β,17-dihydroxyacetoneketal	Beverage (herbal tea), cytotoxic (HCT 116: human colon cancer cell)
Rubus suavissimus	Rubsoside (=steviol-13,19-di-*O*-β-*D*-glucoside) (**19**), stevioside (**20**), rebaudioside A (**25**), suavioside A (**19a**), steviol monoside (**19b**), suaviaside B (**19c**), suaviosaide G (**19d**), suavioside J (**19e**), suavioside H (**19f**), suavioside I (**19g**), *ent*-3-oxo-16β-kaurane-17-*O*-β-glucopyranoside (**19h**), panicloside IV, *ent*-16α,17-dihydroxykauran-19-oic acid	Beverage (herbal tea) Antiangiogenic, antiallergic
Ryonia speciosa	Ryanodine, dehydroryanodine, ryaniaditerpene esters A-D	Calcium transport blocker, insecticide
Salvia aegyptiaca	(*E*,*E*,*E*)-cembrene A	
Salvia aethiopsis	Manool (**34**),15-nor-hydroxy-13-epi-manoyloxide-14-oic acid, salvipisone, 4,5-seco-5,10-friedo-4(18),5(10),6,8,13-arucadiol (=1-ketoaethiopinone)	Antibacterial
Salvia africanalutea	Carnosol (**16**), carnosic acid	Anticancer (breast), anti-inflammatory, antimicrobial, antioxidant, serum triglyceride elevation inhibitory
Salvia amplexicaulis	7-Oxo-abietα-9,12,14-triene, horminone, 7-acetyl-horminone, sugiol, ferruginol	Antihypertensive, cardioactive, vasorelaxant
Salvia anastomosans	Anastomosine	
Salvia apiana	11,12,16-Trihydroxy-8,11,13-abietatrien-20-oic acid (=16-hydroxycarnosic acid)	

Salvia argentea	1*R*-Hydroxy-20-nor-5(10),6,8,13-abieta-tetraene-11,12-dione (=1*R*-hydroxymiltirone), 11,12-dihydroxy-20-*nor*-5 (10),6,8,13-abietatetraen-1-one (=arucadiol), 4,5-seco-5,10-friedo-4(18),5(10),6,8,13-arucadiol (=1-ketoaethiopinone), isopimara-8(9),15-diene, salvipisone, ferruginol, aethiopinone, isocembrene, manool (**34**), sclareol oxide	Foods, basal leaves Acetylcholinesterase and *butyrylcholinesterase* inhibitory, antibacterial, antioxidant, hemostatic, mosquito larvicidal
Salvia atropatana	Atropatanene, 7α-acetoxyroyleanone, saprothoquinone, aethiopinone	Cytotoxic (PC3: prostate cancer cell line)
Salvia austraca	7α-Acetoxyroyleanone, 7α-hydroxyroyleanone	
Salvia ballotaeflora	19,20-Epoxy-7α,12,20-trihydroxy-8,12-abietadiene-11,14-dione, icetexine, romulogarzone	
Salvia barrelieri	Barreliol, royleanone 12-methyl ether, 7-episalviviridinol, iguestrol, ferruginol, taxodione, viridone, demethylinuroyeanol, isomanool, 7-oxo-royeanone-12-methyl ether, 7α-acetoxy-royleanone-12-methyl ether, royleanone, horminone, 7-acetylhorminone, cryptojaponol, inuroylenol	Antioxidant, antibacterial, diuretic, emmenagogue, spasmodic
Salvia bicolor	8,11,13-Abietatriene-7α,11,12-triol	
Salvia bleapharochelana	Psiferic acid derivatives, *O*-methylpisiferic acid, *O*-methylpisiferic acid methyl ester	Antibacterial, antifungal
Salvia bracteata	Bractealine	Antibacterial
Salvia breviflora	*ent*-2β,18-Epoxy-19-hydroxy-3,13-clerodadien-16,15-olide	
Salvia broussonetii	Brussonol	Cytotoxic, insect repellent (sting bag)
Salvia canariensis	7α-Ethoxy-12-hydroxy-8,17-abietadiene-20,6β-olide (=canariquinone), dexoycarnosol methyl ether, rosmaquinone, 7-ethoxyrosmanol, salvicecanaric acid, 7,11,12-trihydroxy-20-*nor*-5,7,9,11,13-abietapentaen-1-one (=arucatriol)	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Salvia candelabrum	Candesalvone, candesalvone A [11, 12, 14-trihydroxy-19 (4→3)-abeo-3, 8, 11, 13-abietatetraen-7-one], candesalvone B [11, 12, 14-trihydroxy-7-oxo-3, 4-seco-4 (18), 8, 11, 13-abietatetraen-3-oic acid] 12,14-trihydroxy-8,11,13-abietatriene-3,7-dione (=candelabrone)	
Salvia candidissima	Candidissiol, manoyl oxide, pachstazone, 20-deoxocarnosol, aethiopinone	
Salvia cardiophylla	Cardiophyllidin, 2,12-dihydorxy-8,11,13-abietarien-7-one (=2α-hydroxysugiol)	
Salvia carnosa	11,12-Dihydroxy-8,11,13-abietatriene-20,7β-olide (=carnosol) (**16**)	
Salvia castanea	Tanshinone II (A), tanshinone II (B), hydroxytanshinone II (A), tanshinone I (**21**), dihydrotanshinone I, cryptotanshinone I, neotanshinone A, neotanshinone B, tanshinone aldehyde, przewaguinone A, przewaguinone B, sugiol	Antioxidant
Salvia chinensis	Salvianol acid, methyl salviano acid, methyl salvianate A, dimethyl lithospermate, salvianoic acid	
Salvia chinopeplica	19-Hydroxyroyleanone, carnosol (**16**), 16-hydroxycarnosol, 16-hydroxy-20-deoxocarnosol, 16-hydroxycanosic acid, 16-hydroxyrosmanol, 11,12,16-trihydroxy-20-*nor*-labdan-5 (7),8,11,13-tetraen-1-one	
Salvia chionantha	Horminone, 7-acetyl horminone	
Salvia chrysephylla (golden leaf sage)	sclareol (**22**)	Antioxidant, acetylcholine esterase and butyrylcholine esterase inhibitory
Salvia cinnabarina	3,4-Secoisopimar-4(18),7,15-triene-3-oic acid	Hypotensive, intestinal spasmolytic activity, intestinal motility

Salvia clevelandii	Rosmadial, 16-hydroxycarnosol, abieta-8,11,13-triene, taxodone, carnasol, rosmanol (**17**), carnosic acid	Anticancer, cytotoxic
Salvia clinopodioides	Clinopodioides A-D	Antilipido-peroxidative, antiamoebic, antigiardial, antioxidant, antipropulsive, antiprotozoal
Salvia coccinea	Clerodatetraene-17,12:18,19-diolide (=salviacoccin)	
Salvia columbariae (Chia)	Tanshinone II A, cryptotanshinone (**45**)	Food (seeds) Cytotoxic
Salvia corrugata	Fruticuline A, C, demethylfruteculine A	Antimicrobial, bacteriostatic
	7α-Methoxy-19-acetoxyroyleanone, 7α,19-diacetoxyroyleanone, 7-dehydroxyconacytone	Chemopreventive
Salvia cryptantha	6,8,11,13-Abietateraene-11,12,14-triol (=cryptanol), 2β,12-dihydroxy-8,12-abietadiene-11,14-dione (=2β-hydroxyroyleanone), fruticuline C, 7α-methoxy-19-acetoxy-royleanone, 7α,19-diacetoxy-royleanone, 7-dehydroxyconacytone	Chemopreventive
Salvia divinorum (psychoactive mint)	Salvadoran A (=divinorum A), B, salvinorin C-G, divinatorins A-D, hardwickiic acid, salvinicin A , B, salvidivin A, C, H	Anti-inflammatory, recreational drug, agonistic activity to opioid receptor For headache, diarrhea, hallucinations
Salvia elegans	Pimara-7,15-dien-3-one, sclareol (**22**), totarol, 13β-methylvinylpodocarp-7-en-3β-ol	Antianxiety, antidepressant, antihypertensive, antioxidant For hypertension
Salvia eremophila	Carnasol, carnosic acid	Beverage (herbal tea), flavoring agent Antimicrobial, antioxidant
Salvia eriophora	Ferruginol, aethiopinone, 1,2-dihydroxysapi-paraquinone, 6,7-didehydroroyleanone, 4,14-dihydroxysaprotho-quinone	Antihypertensive, cardiovascular activity, vasorelaxant
Salvia euphratica	8,11,13-Abietatriene-2α,11,12-triol (=eupharatricol), 11,12,15-trihydroxy-8,11,13-abietatrien-20-al (=euphracal)	
Salvia farinacea	*ent*-10βH-7β,20:12, 20:15, 16-Triepoxy-1,2,13(16), 14-clerodatetraen-18,19-olide	
Salvia forskahlei	Forskalinone, multicaulin	Antibacterial

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Salvia fruticosa	Carnosol (**16**), dehydroabietic acid (**43**), carnosic acid	Antibacterial, antioxidant Infusion: For headache, circulatory system diseases
Salvia fruticulosa	11-Hydroxy-2-methoxy-19,20-dinor-1,3,5(10), 7,9(11),13-abietahexaene-6,12-dione, fruticulin A, demethylfruticulin	Anticholinesterase, antimicrobial, antioxidant
Salvia fulgens	Salvifulgenolide, (*E*)-1,2-dihydro-salvifaricin, *ent*-19-acetoxy-15,16-epoxy-3,13(16),14-clerodatrien-6,18-diol, *ent*-19-acetoxy-15,16-epoxy-6-hydroxy-3,13(16),14-clerodatrien-18-al, 10β-hydroxy-bacchotrieneatin A	Culinary herb Hallucinogenic For stomach ailments
Salvia gilliessi	5-*epi*-Icetexone	Antimicrobial, antiproliferation, antitrypanosomal (*Trypanosma cruzi*)
Salvia glutinosa	12-Deoxydanshenxinkun B, dihydroisotanshinone II, isotanshinone II	Anticholinesterase, antimicrobial, antioxidant
Salvia grandifolia	Grandifolia A-F, tanshinone VI, tetrahydrotanshinone, tanshinone II B, danshenol B, salvianolic acid, nepetoidin B	Hepatoprotective, vasorelaxant
Salvia greggii	Salvigresin, salvigresides A-D, 15,16-epoxy-10-hydroxy-13(16),14-clerodadiene-17,12:18,19-diolide (=7,8β-dihydrosalviacoccin)	
Salvia herbacea	Tehuanin A-H	
Salvia hispanica (chia)	Hispanin A (**166**), B-F, G (**167**), I, J, 12-hydroxyhautriwaic lactone, bacchotricuneatin, rhynchospermoside, (*E*)-1,2-dihydro-salvifaricin, 8-hydroxysalviarin, 12-hydroxyhardwickic acid	Food (seeds) Antidiabetes, anti-inflammatory, cardioprotective For cardiomyocyte injury, cardiovascular diseases, nervous system disorders
Salvia hypargeia	6β,12-Dihydroxy-8,11,13-abietatriene-1,7-dione (=hypargenin A), 12,15-dihydroxy-8,11,13-abietatrien-7-one (=hypargenin B), 12-hydroxy-8,11,13-abietatrien-6,7-dione (=hypargenin C) hypargenins D-F	Antibacterial, antimicrobial

Salvia infuscata	Infuscatin	
Salvia jamensis	15,16-Epoxy-cleroda-3-en-7α,10β-dihydroxy-12,17:19,18-diolide, Isopimaric acid	Increase adenosine-5'-diphosphate induced platelet aggregation inhibitory
Salvia keerlii	7α-Acetoxy-12*R*-hydroxy-*neo*-cleroda-3,13(14)-diene-15,16:18,19-diolide (=kelinolide), 7β,12-dihydroxy-3,13-clerodadiene-15,16:18,19-diolide (=kerlinolide), *ent*-8α,12R-epoxy-3,13*S*(14)-clerodadiene-15,16:18,19-diolide (=kerlin), *ent*-15,16-epoxy-6β-hydroxy-3,13(16),14-cleroda-trien-19-oic acid (=kerlinic acid)	
Salvia kronenburgii	Horminone, 7-acetyl horminone	
Salvia lanata	7α,12-Dihydroxy-8,12-abietadiene-11,14,20-trione (=desacetylnemorone), 7α-acetoxy-12,20-dihydroxy-8,12-abietadien-11,14-dione	
Salvia languidula	Languiduline	
Salvia lanigera	Langierol, 11,14-dihydroxy-8,11,13-abietatrien-20,7-olide	Antimicrobial
Salvia lasiantha	*ent*-10β-Acetoxy-2-oxo-3,13-clerodadien-15,16-olide	
Salvia lavandulifolia	Galdosol	Beverage (herbal tea, liqueur), condiment Antimicrobial, antioxidant, cytoprotective
Salvia leriaefolia	8(17),12*E*,14-Labdadien-6,19-olide	Antimicrobial
Salvia leucantha	Salvoleucalon B	Cytotoxic
Salvia limbata		Antioxidant, antiviral
Salvia lineata	*ent*-15,16-Epoxy-1,3,13(16),14-clerodatetraene-17,12:18, 19-diolide (=linearifolin), *ent*-15,16-epoxy-1(10),2,13(16),14-clerodatetraene-17,12:18, 19-diolide (=dehydrosalvianin)	
Salvia macrophylla	Corsonic acid, salvimacrophyllin A-D, 17-methoxycarnosic acid, 7-oxo-8(14),15-iso-pimaradien-18-oic acid (=7-oxosandaracopimaric acid), 7-oxo-sandaradomimaric acid methyl ester	Antimicrobial
Salvia macrosiphon	13-epi-Manoyl oxide, savigenin	Cytotoxic, laxative For cough, sore throat

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Salvia melissodora	Melisodoric acid, *ent*-2α,7β-dihydroxy-3,13-clerodadiene-16,15:18,19-diolide, *ent*-2α-acetoxy-7β-hydroxy-3,13-clerodadiene-16,15:18,19-diolide, *ent*-2α-hydroxy-7β-acetoxy-3,13-clerodadiene-16,15:18,19-diolide, *ent*-2α-acetoxy-7-oxo-3,13-clerodadiene-16,15: 18,19-diolide, 7α,18,19-trihydroxy-3,13-clerodadien-16,15-olide, *ent*-6β-hydrocy-3,13-clerodadien-15,16-olide-19-oic acid	Antifeedant
Salvia miltiorrhiza	Tanshinone I (**21**)	Anti-inflammatory, apoptosis (MCF-7: breast cancer, MDA-MB-231: estrogen receptor negative estrogen receptor positive), cytotoxic (MCF-7, MDA-MB-231 cancer cell lines) NO production inhibitory Apoptotic inducing effect (rat primary cortical cells)
	Tanshinone I (**21**), tanshinone II, tanshinone II B, tanshinoic acid, militibetin A, yunnannin A, ferruginol, miltionones II, 15,16-dihydrotansinone I	Enhance activity of insulin on the tyrosine phosphorylation of the insulin receptor (RI) β-subunit Cytotoxic (HONE-1, HT-29, P388 cancer cell line) For angina, heart attack, stroke
	Sibiriquinone A, B cryptotanshinone (**45**)	Anticancer (HIF-1: gastric, Hep 3B human cancer cell lines)
	Tanshinone II A (**168**)	Apoptotic inducing effect on a large variety of cancer cells, osteoclastogeneis inhibitory
	Cryptotanshinone (**45**) Tanshinone II B, tanshinone lactone A	Anti-Alzheimer's disease, antiproliferative, immunomodulatory
	8,11,13-Abietatriene-2α, 12-diol (=salviol), 2α-hydroxyferruginol), 7β-hydroxy-8,13-abietadiene-11,12-dione, 11-hydroxy-8,11,13-abietatrien-7-one (=sugiol), 12,6-epoxy-20-*nor*-5,7,9,12,15-abietapentadiene-11,14-dione, danshenspiroketal lactone, 13-epidanshen-spiroketal lactone,	

	3,16-dihydroxy-2-isopropyl-8-methyl-1,4-phenanthraquinone (=danshexinkun A), danshexinkun B, C, 1,2-dihydro-1,6-dimethylfuro[3,2-c] naphtha[2,1-e]-oxepine-10,12-dione, formyltanshinone, isotanshinone I, methylenedihydrotanshin-quinone, 4-methylenemiltirone, miltinone I, miltinone II, miltirone, 1,5,7,9,13-abietapentaene-11,12-dione (=didehydromiltirone), neocryptotanshinone, tanshindiols A-C, tanshinlactone, 1,2,15,16-tetrahydro-tanshinone I, tanshinone II, 3α-hydroxytanshinone II A, tanshinone II B, tanshinonic acid methyl ester, 6,7,8,9-tetrahydro-1,6,6-trimethylfuro[3,2-c] naphtha[2.1-e]oxepine-10,12-diene, salviolone, 7β,20-epoxy-13-methyl-8,11,12-podocarpatrien-12-ol (=norsalvioxide)	
Salvia moorcrofiana	11β-Hydroxy-5,7,9(11), 13-abietatetraen-12-one, 11-hydroxy-5,7,9(11),13-abietatetraen-12-one (=deoxyfuersitone)	
Salvia multicaulis	12-Demethylmulticaulin, multiorthquinone, 2-demethyl-multiorthquinone, 12-methyl-5-dehydro-acetylhorminone, salvipinarone	Antibacterial, anticholinesterase, antioxidant
	12-Methyl-5-dehydrohorminone	Antifungal
Salvia nemorosa	7,12-Dihydroxy-8,12-abietadiene-11,14,20-trione (=desacetylnemorone)	
Salvia officinalis	Carnosol (**16**), 12-*O*-methyl carnosol, isorosmanol (**18**), columbaridione, carnosic acid (**169**), 12-*O*-methylcarsonic acid, rosmanol (**17**), epi-rosmanol, galdosol, atunzensin A, rosmadial, miltirone, (*5S,6S,7S,10R,12S,13R*)-7-hydroxyapiana-8,14-diene-11,16-dion-(22,6)-olide, (*5S,6S,7S,10R,12R,13S*)-7-hydroxyapiana-8,14-diene-11,16-dion-(22,6)-olide, (*5S,6S,7S,10R,12R,13S*)-7-hydroxyapiana-8,14-diene-11,16-dion-(22,6)-olide, rosmanol-7-ethyl ether, 6,7-O-dimethyl-epi-rosmanol (**170**), abietane-7-oxoroyleanone-12-methyl ether, 7-acetoxyroyleanone-12-methyl ether, royleanone, horminone, 7-acetylhorminone,	Foods, food flavor (sausage, pickles, soup, canes, vine), beverages (herbal tea), crude drugs, spice Anthelmintic, antibacterial, antifungal, anti-inflammatory, antioxidant, antiobesity, antisecretary, antiseptic, antispasmodic, antiviral, astringent, carminative, central nervous system (CNS) activity, cytotoxic (Caco2: colonic carcinoma, HepG2; hepatoma cancer cell lines), diuretic, emmenagogue, gastroprotective, hemostatic, hypoglycemic, insect repellent, NO production and NF-κB transcription factor inhibitory, sedative, strengthen bones and

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
	cryptojaponol, inuroyleanol (=7-oxo-11,14-dihydroxy-12-methoxy-8,11,13-abietatriene, 20-norabietanes: safficinolide (**171**), sageone (**172**), 7-methoxyrosmanol, galdosol, royleanone, horminone, acetylhorminone, manool (**34**)	immune system, tonic, tranquilizer, wound healing For cough, hot infusion: for bronchitis, stomachic
Salvia oxodon	3α-Hydroxy-dehydroabietic acid, 3β-acetoxy-abieta-8(14)-en-18-oic acid 9α,13α-endoperoxide, 3β-hydroxyabieta-8(14)-en-18-oic acid 9α,13α-endoperoxide	
Salvia pachphylla	Carnasol, 20-deoxocarnasol, carsonic acid, iso-rosmanol (**18**), 7-methoxyrosmanol, 5,6-didehydro-*O*-methylsugiol, 8β-hydroxy-9(11),13-abietadien-12-one, 11,12-dioxoabieta-8,13-dione, 11,12-dihydroxy-20-*nor*-abieta-5(10),8,11,13-tetraen-1-one, pachyphyllone	
Salvia pachstachys	2,12-Dimethoxy-8,11(13)-abietatriene (=salvinol dimethyl ether)	
Salvia phlomoides	11,12-Dihydroxy-8,11,13-arbietatrien-7-one (=demethyl-cryptojaponol), 6α,7β-dihydroxy-8,13-abietadiene-11,12-dione, 6,11,12trihydroxy-5,8,11, 13-abietatetraen-7-one	
Salvia plebeia	Plebeianiol, carnosol (**16**), isocarnosol, saficinolide (**171**), 2,11,12-trihydroxy-7,10-epoxy-8,11,13-abietatriene, 2β,3β:18,19-diepoxy-10-hydroxy-7,13(16),14-clerodatriene-17,12:18,19-diolide	Anti-inflammatory, antimicrobial, antioxidant, antitumor, urinary tract infection For bronchitis
Salvia polycephala	20-Hydroxy-3,13*Z*-clerodadien-15-oic acid (=stephalic acid)	
Salvia polystachia	linearolactone	Antiprotozoa
Salvia pomifera		Antioxidant

Salvia pratensis		Antimicrobial, antioxidant
Salvia prionitis	4-Hydroxy-saprorthoquinone, 3-keto-4-hydroxy-saprorthoquinone, 11,12-dihydroxy-5,8,11,13-abietatetraen-7-one (=salvinolone), 4,5-seco-5,10-friedo-3,5,6,8,13-abietapentaene-11,12-dione (saprorthoquinone), sapriparaquinone, 3-oxo-sapriparaquione, 4-hydroxysapripara-quinone, pionitin, salvinolacone	Cytotoxic (HL-60: leukemia, SGC-790 and MK-28): stomach cancer cell lines
Salvia przewalskii	Tanshinone II, Cryptotanshinone (**45**), 18-deoxy-3α-hydroxy-tanshindiol A (=tanshinodiol B), 18-deoxy-3β-hydroxy-tanshindiol A (=tanshinodiol C)	Induce CYP3A4 at transcriptional level, by activating human pregnane X-receptor on HepG2 cells, vasorelaxant
Salvia puberuda	Isosalvipuberulin	
Salvia pubescens	19*R*-acetoxy-19-deoxoicetexone, 3β,11,12-trihydroxy-8,11,13-abietatrien-7-one (=3β-hydroxydemethylcryptojaponol), 19(4→3)abeo-11,12-dihdyroxy-4(18)-8,11,13-abietatetraen-7-one	Antibacterial
Salvia recognita	12-Methyl-5-dehydro-horminone	Antifungal
Salvia reflexa	8-Hydroxysalviarin, 7,8-didehydrorhyacophiline, 15,16-epoxy-8α-hydroxy-*neo*-cleroda-2,13(16),14-triene-17,12*R*:18,19-diolide, 7,8-didehydrorhyacophiline, salviarin, 6β-hydroxysalviarin	Antioxidant
Salvia regla	7α,12,20-Trihydroxy-11, 14-dioxo-8,12-abietadien-19-oic acid (=diacetyl sessein)	
Salvia rhytidea	1-Deoxoaurocadiol, ferruginol, taxodione, arucadiol, deoxyneocryptotanshinon, 7α-ethoxyroyleanone, microstegiol, 12-hydroxysapri-paraquinone	Antibacterial, antifungal, anti-inflammatory, antioxidant, antiproliferative, anti-tumor, cytotoxic For digestive and healing

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Salvia rosmarinus (=*Rosmarinus officinalis*)	Carnosol (**16**), rosmanol (**17**), isorosmanol (**18**), 7-methoxyrosmanol, epi-rosmanol, rosmadial, cryptotanshinone (**45**), carnosic acid, methyl carnosate, 7-methoxy-*epi*-rosmanol	Aerial part: Flavoring food, cosmetic, perfume, spice, spray, crude drug, beverages (herb tea) Anticancer, anticarcinogenic, Antidepressant, antidiabetes, anti-inflammatory, antimicrobial, antiobesity, antioxidant, antiseptic, antispasmodic, antiulcerogenic, carminative, GI irritant, hepatoprotective, neuroprotective, neurotrophic, acetylcholine stevase inhibitory For brain aging and muscular degradation protection, enhancing memory and concentration, hair growth, hepatic, improving digestion, intestinal renal, neurological, respiratory affections, skin health
Salvia santolinifolia	Carnasol, carnosic acid	Beverage (herbal tea), flavoring agent Antibacterial, antioxidant hemorrhoids, hypercholesterolemia For diarrhea
Salvia sapinae	8β,11,13-Abietatriene-7,15-diol	
Salvia sclarea	Ferruginol, salvipisone, microstegiol, candidissiol, 2,3-dehydrosalvipisone, aethiopinone, 1-oxo-aethiopinone, salvinolone, cryptojaponol, Δ^7-manool, 14-Labdene-8α,13*S*-diol (=sclareol (**22**)), manool (**34**), dehydrosalvipisone, 7-oxoroyleanone	Food flavoring, salads, beverages (herbal tea) Antibacterial, antidepressant, anti-inflammatory, antispasmodic, antiviral, appetizer, aromatic, astringent, bactericidal, bacteriostatic balsamic, carminative, tonic For circulation, indigestion, kidney disorders, pectoral, pain and pre-menstrual problems, stomach, stress relief
Salvia semiatrantha	2α-Hydroxy-3,13-clerodadiene-15,16:18,19-diolide (=semiatrin)	

Salvia sessei	7α-Acetoxy-12,20-dihydroxy-11,14-dioxo-8,12-abietadien-19-oic acid (=sessein)	
Salvia shannoni	Sepulturin A-F, infuscatin	Anti-inflammatory antiprotozoal, cytotoxic
Salvia splendens	Salvinolin A, *ent*-15,16-epoxy-2,13(16),14-clerodantriene-17,12: 18,19-diolide (=salviarin), *ent*-15,16-epoxy-10α,11-dihydorxy-2,13(16),14-clerdatriene-17,12:18,19-diolide	Antifeedant
Salvia staminea	Manoyl oxide	Antimicrobial
Salvia syliaca	Ferruginol, candidissiol, 4-dehydrosalvilimbinol, viridone, salvisyrianone	Antihypertensive, vasorelaxant
Salvia texana	6,8,11,13-Abietatetraene-2α,12-diol (=6,7-didehydroalviol), 2α,11-dihydroxy-5,7, 9(11),12-abietatetraen-12-one (=6-deoxo-2α-hydroxy-taxodione), 11-hydroxy-7,9(11),13-abietatrien-2,6,12-trione (=2-oxo-taxodione), 2α,6,7,11-tetrahydroxy-5,7,9(11),13-abietatetraen-12-one (=5,6-didehydro-2,7-dihydroxytaxodione), 2α,6α,7,11-tetrahydroxy-7,9(11),13-abietatrien-12-one (=2α,7-dihydorxytaxodione, 2α,6α,11-trihydroxy-7,9(11),13-abietatrien-12-one (=2α-hydroxytaxodione), 2α-hydroxysalvicanaric acid, salvicanaric acid, 2α,11,12-trihydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial-11,6-hemiacetal	
Salvia tilifolia		Anticholinesterase, antioxidant
Salvia tiloaefolia	Isosalvipuberulin, trifodiolide	
Salvia tomentosa	7α-Acetoxyroyleanone, 7α-dihydroxyroyleanone, 6,7-dehydroxyroyleanone, 3β-hydroxydehydroabietic acid (=3β-hydroxy-8,11,12,15-abietatetraen-18-oic acid)	Beverage (herbal tea), condiment (leaf) Antimicrobial, antioxidant
Salvia verticillata (purple sage, lilac sage)	7α-Acetoxyroyleanone, 7α-hydroxyroyleanone	Antibacterial, antioxidative For anxiety, depress in epilepsy

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Salvia viridis	*epi*-13-Manoyl oxide	Antibacterial, antifungal, antioxidant, antiviral
Salvia wiedemanii	8,11,13-Abietatrien-3-one, 9α-hydroxy-7-oxo-15-abieten-18-oic acid	
Salvia yunnanensis	Yunnanin A, danshenol, 7,8-seco-para-ferruginone	Antimicrobial, antitumor (BIU87: bladder, K-562: erythromyeloblastoid, Me-180: cervical, T-24: bladder carcinoma cell lines)
Sargassum macrocarpum (algae)	Tuberatolide B (TTB)	
Sargassum micracanthum	2-Geranyl geranyl-6-methyl-1,4-benzoquinone	Antioxidant, cytotoxic (colon 26-1.5 cell line)
Sargassum siliguastrum (algae)	sargachromenol E	
Satureja hortensis (savory)	Carnosol (**16**), carnosic acid	Antibacterial, antidiarrheal, anti-inflammatory, antinociceptive, antioxidant, antispasmodic, hepatoprotective insecticidal, pesticidal, sedative
Satureja montana		Beverage (herbal tea, liqueur), spice Antibacterial, antifungal, antimicrobial, antioxidant, cytotoxic
Sceptrum labdalactone		Antimicrobial
Sciadopitys verticillata	15,16-Dihydroxy-8(17),13*E*-labdadien-19-oic methyl ester (=sciadopic acid methyl ester), 12S,20:15,16-diepoxy-8(17),13(16), 14-labdtrien-19,20-olide (=sciadin), *ent*-15-kaurene, *ent*-kaur-16-ene, 15-phyllocladene (=isophyllocladene), verticillol	
Scoparia dulcis	Dulcinol, scopadulcic acid A, 4-*epi*-scopaduli acid A (=scopadulic acid B)	
Scutellaria alpina	Scutalpin	

Scutellaria baicalensis	Scutebaicalin (**173**)	Antifungal, anti-inflammatory, antioxidant, cardiovascular, insect antifeedant, kidney, liver diseases
Scutellaria galericulata	*ent*-2β,19:18,11,16:15,16-Tetraepoxy-14-clerodene-1α-tigloyl-19-acetoxy-6β-ol (=jodrellin T), *ent*-2β,19:18,11,16:15,16-tetraepoxy-14,15-dihydroclerodene-1α-tigloyl-19-acetoxy-6β-ol (=dihydrojodrellin T), 4,18:11,16:15,16-triepoxy-3α-tigloyl-6β,19-diacetoxy-14-cleroden-2α-ol (=galericulin)	
Scutellaria indica		Anticancer, anti-inflammatory antioxidant, antipyretic, antitumor
Scutellaria rivularis	3β,4β-Epoxy-6β-benzoyl-8-hydroxy-11,13-clerodadien-15,16-olide (=secutellone F), *ent*-8,13-epoxy-3α,4α-dihydroxy-6β-benzoyl-11α-acetoxy-15,16-clerodanolide, *ent*-3α,4α,8α-trihydroxy-6β-benzoyl-11*E*,13-clerodadien-15,16-olide (=scuterivulactone D)	
Scutellaria woronowii	*ent*-2β,19:4β,18:11*R*, 19*S*:15,16S-Tetraepoxy-14-cleroden-6β,19-diacetate (=jodrellin A), *ent*-2β,19:4β,18:11*R*,19*S*:15,16S-tetraepoxy-6β-acetoxy-19-(2-methyl-propanoyl)-14-clerodene (jodrellin B)	
Scyopholepia hookeriana	*ent*-8(14)-Pimarene-3β-*O*-[3-*O*-methyl-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside], 12β-(3-*O*-methyl-β-*D*-quinovopyranoside (=hookeroside A), *ent*-8(14)-pimaradiene-3β-*O*-[3-*O*-methyl-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside], 12β-*O*-β-(fucopyranoside (=hookeroside B), *ent*-8(14)-pimaradiene-3β-*O*-[β-*D*-fucopyranosyl (1→2) β-*D*-fucopyranosyl (1→2)-α-*L*-arabinofuranoside], 12-*O*-β-D-fucopyranoside (=hookeroside C), hookeroside D	
Selaginella denticulate	*ent*-Labda-7,13(*E*)-dien-15-ol	Beverage (herbal tea) Antihypertensive For cough

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Sequioia sempervirens	20-Hydroxyferruginol, 6α-hydroxysugiol	Anticancer (breast, colon, lung)
Sida rhombifolia	Some diterpenoids	Decoction: for cleansing open sores Infusion: for dysentery Cytotoxic, antibacterial
Sideritis angustifolia	*ent*-15-Beyerene-7α,12α, 17-triol (=conchitriol), *ent*-13-atisene-7α,16α,17-triol (=isosideritol)	
Sideritis arborescens	*ent*-16-Kaurene-11β,18-diol	
Sideritis asora	7α,18-Dihydrokaur-16-ene	Antifungal
Sideritis biflora	*ent*-15β,16β-Epoxy-3β,7α,18-kaurenetriol	
Sideritis canariensis	*ent*-7α,18-Trachylbanediol	
Sideritis candicans	*ent*-Kaurene-7β,18-diol (=candicandiol), *ent*-kaurene-7α,18-diol (=*epi*-candicandiol), *ent*-kaurene-18-acetoxy-7β-ol, 17-nor-7α,18-dihydroxy-*ent*-kaur-16-one (=adejone), *ent*-16-kauren-7α-ol (=candol *A*), *ent*-16-kauren-18-ol (=candol *B*), *ent*-7α-acetoxy-16-kauren-18-ol, epicandicandiol	Beverage (herbal tea) For bronchitis, cough, digestive, intestinal diseases
Sideritis chamaedryfolia	8β,15,19-Trihydroxy-13-labden-7-one (=villenatriolone), 16-kaurene-7α,18-diol (=*epi*-candicandiol)	
Sideritis condensata	Linearol, isolinearol, siderol, sideridiol, sideloxol, 7-acetylsideroxol, candol B	Beverage (herbal tea) Acaricidal, insecticidal
Sideritis funkina	*ent*-15-Kaurene-3α,6α,7α,18-tetrol (=sidofunkiol)	
Sideritis leptoclada	Sideroxol, *ent*-7α,18-dihydroxy-15β, 16β-epoxykaurene	Antimicrobial, anticancer, malignant melanoma, antioxidant
Sideritis leucantha	*ent*-15-Kaurene-3β,7α,17, 18-tetrol (=isoleucanthol), 16-kaurene-3,7,15,18-tetraol, *ent*-15-kaurene-3β-acetoxy-7α,18-diol (=isosidol), foliol, sidol	Antioxidant

Sideritis linearifolia	Foliol, sidol	Antioxidant
Sideritis lycia	Linearol, 7-epicandicandiol, siderol, sideridiol, sidol	Anticancer, cytotoxic
Sideritis niveofomentosa	Siderol, sideridiol, 7-epicandicandiol, sidol, eubotriol, eubol, anthonolone, linearol	Anti-inflammatory, antimicrobial, antioxidant, antirheumatism, antiseptic, insecticide For cold, cough, gastrointestinal disorders
Sideritis paulii	*ent*-15α,16α-Epoxy-kaurene-3β,7α,18-triol	
Sideritis perfoliata	13-*epi*-Manoyl oxide	Antimicrobial
Sideritis pullulans	1α,3α,7β,18-Tetrahydroxy-*ent*-kaur-16-ene (sideripullol A), 3α,11α,18-trihydroxy-*ent*-kaur-16-ene (sideripullol B), 3α,7β,18-trihydroxy-17-*nor*-*ent*-kauran-16-one (sideritone A), 3α,7β-dihydroxy-18-acetyloxy-17-*nor*-*ent*-kauran-16-one (sideritone B), 3α,7β,16α,17-tetrahydroxy-18-acetyloxy-*ent*-kaurane (sideripullol C), 7β,16α,17,18-tetrahydroxy-*ent*-kaurane (sideripullol D)	Cytotoxic (HeLa, PC-3)
Sideritis pusilla	15-Beyerene-7,14,18-triol (=pusillatriol), 13-atisene-11,16,17-triol	
Sideritis raeseri	Bayerene, pimara-8(14),15-diene, *ent*-15-kaurene, manoyl oxide	Beverage (herbal tea)
Sideritis scardica	Beyerene, pimara-8(14),15-diene, *ent*-15-kaurene, manoyl oxide	Beverage (herbal tea) Anti-inflammatory, antioxidant, cytotoxic, expectorant, gastroprotective, immunostimulant, pulmonary emphysema, urogenital diseases
Sideritis serrata	*ent*-15-Beyerene-7α,17-diol, *ent*-15-beyerene-12α,17-diol (=tobarrol)	
Sideritis sicula	*ent*-15-Kaurene-7α-hydroxy-18-acetate (=sideripol)	
Sideritis sipylea	Epoxyisolinearol	Antifungal
Sideritis sp.	Royleanone, linearol, foliol, siderol, 7-*epi*-candicandiol	Antifungal

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Sideritis stricta	*ent*-1β-Hydroxy-7α-acetoxy-15β,16β-epoxykaurane (**174**), sideroxol, 7-*epi*-candicandiol, linearol, *ent*-7α,15β,18-trihydroxykaur-16-ene, *ent*-7α-acetoxy-15,18-dihydroxy-kaur-16-ene, foliol, sideridiol, siderol 2,3-dehydrosalvipinone, 7-oxo-royleanone epoxyisolinearol	Antibacterial, antifungal
Siegesbeckia orientalis	*ent*-12α,16-Epoxy-2β,15α, 19-trihydroxypimar-8-ene, *ent*-12α,16-epoxy-2β,15α, 19-trihydroxypimar-8(14)-ene, *ent*-2α-15,16,19-tetrahydroxypimar-8(14)-ene,*ent*-15-oxo-2β,16,19-trihydroxypimar-8(14)-ene, *ent*-2-oxo-15,16-dihydroxypimar-8(14)-en-16-*O*-β-*D*-glucopyranoside, *ent*-2-oxo-15,16,19-trihydroxypimar-8(14)-ene, *ent*-2-oxo-3β,15,16-trihydroxypimar-8(14)-en-3-*O*-β-*D*-glucopyranoside, *ent*-2β,15,16,19-tetrahydroxypimar-8(14)-en-19-*O*-β-*D*-glucopyranoside, *ent*-8,13-pimarene-3β-*O*-β-*D*-glucopyranosyl-15*S*,16-diol	Food (young leaves) For hypertension, malaria, neurasthenia, rheumatic arthritis, snake bite
Siegesbeckia pubescens	*ent*-8(14)-Pimarene-2α,15,16,19-tetrol (=kirenol), *ent*-8(14)-pimarene-6β,15,16,19-tetrol, *ent*-16β,17-dihydroxykauran-19-oic acid	Chinese folk medicine Antithrombic
Silphium perfoliatum	8α,12*R*-Epoxy-13*E*-labdene-15,16-diol (=16-hydroxycarterochaetol)	
Sinularia flexiblis	1*R*,3*S*,4*S*-Epoxy-11*R*-hydroxy-7*E*,15(17)-cembradien-16,12*R*-olide (11-*epi*-sunulariolide), 1*R*,3*S*,4*S*-epoxy-11*S*-hydroxy-7*E*,15(17)-cembradien-16,12*R*-olide (=sunulariolide), 2,6,6,9,13-pentamethyl-1,4,8,12-cyclopentadecatetraene (=flexibilene)	

Sinulasria mayi	2*E*,4*Z*,7*E*,11*E*,15-Cembrapentaene (=cembrene), (1*R*,2*R*,3*E*,7*E*,11*E*,15-cembratetraen-2-ol (=mayol)	
Solenostemon monostachys	2-*O*-Acetyl-17-acetoxy-15-epicoleon Z, 12-*O*-acetyl-15-epicoleon *Z* (=mon A), mon B, mon C	
Solenostemon sylvaticus	12-*O*-Acetyl-17-acetoxy-15-epicoleon *Z*, 12-*O*-acetyl-17-formyl-15-epicoleon *Z*, coleon Z, 6-*O*-acetyl-17-acetoxy-12-*O*-desacetylcoleon P (=syl A), 19-acetoxycoleon O (=syl C), coleon *Z*, 2-*O*-desacetyl-7-*O*-acetyl-19-acetoxycoleon P	
Solidago altissima	*ent*-1β-Acetoxy-3,13-clerodadien-15-oic acid (=solidagonic acid), 6β-hydroxy-3,13-clerodadiene-15,16-olide (=5α,6β,8β*H*-elongatolide, 3β,4β-epoxy-6-angeloyl-13-cleroden-15,16-olide (=elongatolide E), 3β,4β-epoxy-6-tigloyl-13-cleroden-15,16-olide (=solidagolactone VIII)	
Solidago arguta	*ent*-15,16-Epoxy-3,13(16)-,14-clerodatriene, 15,16-epoxy-3,13(16)14-clerodatrien-18,6β-olide	
Solidago canadensis	15,16-Epoxy-9α-hydroxy-7,13(16),14-labdatrien-6-one (=solidagenone)	
Solidago elongata	6β-Hydroxy-3,13-clerodadiene-15,16-olide (=elongatolide A, 6β-acetoxy-3,13-clerodadiene-15,16-olide (=elongatolide B), 6β-angeloyl-3,13-clerodadiene-15,16-olide (=elongatolide C), D	
Solidago juncea	*ent*-15,16-Epoxy-3,13(16),14-clerodatrien-20-oic acid (=junceic acid), 8(14)-abietene-7α,13-ol (=junceanol V), 8 (14)-abietene-6α-(3-ethyl-2-butenyl)-7α,13-diol	
Solidago leongata	6β-Hydroxy-3,13-clerodadien-15,16-olide (=elongatolide B)	
Solidago missouriensis	*ent*-7,13-Abietadiene, 7,13-abietadien-2β-ol, (+)-*ent*-7,13-abietadien-3-one, (+)-*ent*-7,13-abietadien-3α-ol, 8(14)-abietene-3-oxo-13β-ol (=missourienol) A, 8(14)-abietene-3β,13β-ol (=missourienol) B	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Solidago nemoralis	*ent*-20-Oxo-3,13-clerodadien-16,15-olide (=rugosolide), *ent*-7,13-abietadien-18-oic acid (=*ent*-abietic acid), *ent*-7,13-abietadien-5α-ol	
Solidago serotina	*ent*-15,16-Epoxy-3,12(16),14-clerodatrien-19-oic acid, solidanoic acid A, *ent*-15,16-epoxy-18-hydroxy-3,13(16),14-clerodatrien-19-oic acid (=solidagoic acid B)	
Solidago shortii	*ent*-3α,4α-Epoxy-13-cleroden-15,16-olide, *ent*-3α,4α-epoxy-16-hydroxy-13-cleroden-15,16-olide	
Solidago virgaurea	Clerodanes	Antimicrobial
Spinacea olerocea	Gibberellin A_{53} (**175**)	Vegetable For fatigue, intestinal and stomach complaints, stimulating growth in children, recovery from illness
Stachys aegyptiaca (Egyptian mountain tea qourtom)	Stachaegyptin A, F-H, stachysperoxide	Beverage (herbal tea) Antifungal, antimicrobial
Stachys affinis		Food tonic
Stachys alopecuros		Antibacterial antifungal
Stachys anisochila	Abietatriene	Antifungal, antimicrobial, antioxidant
Stachys annua	*ent*-7β,13-Dihydroxy-3,14-clerodadien-2-one (=stachysolone), *ent*-7β,13-diacetoxy-3,14-clerodadien-2-one	Anti-inflammatory, hepatoprotective
Stachys atherocalyx		Beverage (herbal tea) Anti-inflammatory
Stachys balansae		Beverage (herbal tea) Antibacterial, antifungal
Stachys bayburtensis		Beverage (herbal tea) Antibacterial, antifungal

Stachys bithynia		Beverage (herbal tea) Antibacterial, antifungal
Stachys byzantina	Phytyl nonadecanoate	Beverage (herbal tea) Anticancer, anti-inflammatory For ulcers Infusion: for antiasthmatic, circulatory system diseases, varicose veins
Stachys candida		Antimicrobial
Stachys carduchrorum		Beverage (herbal tea) Antibacterial, antifungal
Stachys cretica subsp. *anatorlica*		Beverage (herbal tea) Antibacterial, antifungal
Stachys cretica subsp. *bulgarica*		Antibacterial, antifungal
Stachys euboica		Beverage (herbal tea) Antifungal, antimicrobial
Stachys floridana (yinmaiao)		Food Antifungal
Stachys geobombycis		Food Tonic
Stachys germanica	Abieta-8,11,13-triene	Leaves, flower-tops (herbal tea) Antifungal, anti-inflammatory, antimicrobial, antioxidant, antiproliferative, cytotoxic For ulcers
Stachys germanica subsp. *heldreichii*	Abietatriene, *ent*-laurenene	Beverage (herbal tea) Antibacterial, antifungal
Stachys glutinosa	Dehydroabietane	
Stachys hubermorathii		Beverage (herbal tea) Antibacterial, antifungal
Stachys huetii		Beverage (herbal tea) Antibacterial, antifungal

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Stachys inflata		Beverage (herbal tea) Anti-inflammatory, infective and rheumatic disorders
Stachys iva	Isoabienol, sclareol (**22**), *cis*-totarol, methyl neoabietate, labda-13(*E*)-en-8α,15-diol	Beverage (herbal tea) Antimicrobial, antioxidant
Stachys lavandulifolia		Beverage (herbal tea) For cold, cough, stomach ailment
Stachys longispicata		Beverage (herbal tea) Antibacterial antifungal
Stachys mentifolia	Abietatriene, abieta-8,11,13-trien-7-one, 13-epi-manoyl oxide, labd-13-en-8,15-di-ol, 16-kaurene	Antibacterial, antifungal
Stachys obliqua		Beverage (herbal tea) Antibacterial, antifungal
Stachys officinalis (=*Betonica offocinalis*)	Betonicoside A-D,	Beverage (herbal tea) Antibacterial, antifungal
Stachys palustris	Abietol, abietatriene, stachysic acid, sclareol, 6β-hydroxy-*ent*-kaur-16-ene, 6β,18-dihydroxy-*ent*-kaur-16-ene, kaur-16-ene, 13-*ent*-manoyl oxide, stacylone, stachone, 13-*epi*-manoyl oxide	Food (leaves, tubers), beverage (seed, herbal tea) Antibacterial, antifungal, antimicrobial, antioxidant, antiradical, antiseptic, antispasmodic, cytotoxic, emetic, emmenagogue, expectorant, homeostatic, nervine, sedative, tonic, vulnerary agent For anxiety, headache, menopausal problems, nervous tension
Stachys parviflorum (=*Phlomidoschema parviflorum*)	Stachysrosane, stachyssaponin A, B, parviflorosides A, B	Antidiarrheal, muscle relaxant, sedative
Stachys persica	Sclareol (**22**)	Leaves, flower-tops: Antifungal, antimicrobial anti-inflammatory, antioxidant

Stachys pinetorum		Beverage (herbal tea) Antibacterial, antifungal
Stachys plumosa	6-Deoxyandulusol, 13-epi-jbugodiol, dehydroabietane, kaurene, biformene, pimaradiene, 3*Z*-cembrene A, manoyl oxide, thumbergol, totarol, abieta-2,11,13-trien-7-one, dehydroabietol, 13-epi-manoyl oxide, sclareol (**22**), abieta-8,11,13-triene, dehydroabietal, methyl neoabietate, dehydroabietane, labd-13*E*-en-8α,15-diol	Leaves, flower-tops: Beverage (herbal tea) Antioxidant, antifungal, antimicrobial
Stachys pumila	Kaur-16-ene, manoyl oxide	Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial, antioxidant
Stachys recta	11α,18-Dihydroxy-*ent*-kaur-16-ene, kaur-16-ene, stachysolone diacetyl and monoacetyl derivative	Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial, antioxidant
Stachys scardica	Abieta-8,11-13-triene, dehydroabietol, sclareol (**22**), dehydroabietal, dehydroabietic acid methyl ester	Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial, antioxidant
Stachys schtschegleevii		Tea Cancer, ulcers, anti-inflammatory, antibacterial
Stachys sericantha		Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial
Stachys setifera		Leaves, flower-tops: Beverage (herbal tea) Antifungal, anti-inflammatory For cancer, genital tumors, ulcers
Stachys spectabilis	Cembrene	Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial
Stachys spinulosa		Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial, antioxidant
Stachys sylvatica	Abietol, abietatriene, stachysic acid, sclareol, 6β-hydroxy-*ent*-kaur-16-ene, 6β,18-dihydroxy-*ent*-kaur-16-ene, kaur-16-ene, 13-*epi*-manoyl oxide, stacylone, stachone, betolide	Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial, anti-inflammatory, Antioxidant, antispasmodic

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Stachys thirkei		Leaves, flower-tops: Beverage (herbal tea) Antifungal, antimicrobial
Stachys tmolea		Beverage (herbal tea) Antifungal, antimicrobial
Stachys viticina		Beverage (herbal tea) Antifungal, antimicrobial
Stachys yemenensis		Beverage (herbal tea) Antifungal, antimicrobial
Stemodia maritima	Stemolide	
Stenochlaena palustris	Phytol, phytadienes	Food, raw as salad or cooked, soup Antibacterial For fever, skin diseases, stomachache, ulcers
Stevia aristata	*ent*-12β-Hydroxy-15-beyeren-19-oic acid, *ent*-7α-hydroxy-15-beyeren-19-oic acid	
Stevia eupatoria	12-Hydroxy-9(11),16-kauradien-19-oic acid, 12β-ethoxy-*ent*-kaur-9(11),16-dien-19-oic acid	
Stevia lineata	15,16-Epoxy-1,3,13(16)-clerodatetraene-17,12;18, 19-diolide (=*ent*-8β*H*, 12α*H*)-form, sterebin E, 11*E*,13*E*-labdadien-6α,7β,8α,15-tetraol, 8α,11*E*, 13*E*-labdadien-6α,7β,8α, 15-tetraol (=6α,7β-brebin F)	
Stevia lucida	*ent*-Kaurenoic acid, stevionolide	
Stevia monadaefolia	6α-Angeloyloxynidrellol	
Stevia ovata	Paniculoside IV	
Stevia paniculata	Paniculosides I-V, crispiloside B	

Stevia phelbophylla	Stevioside (**20**)	Antihypertensive, anti-inflammatory, organoleptic
Stevia polycephala	stephalic acid (=20-hydroxy-3,13-clerodadien-15-oic acid	
Stevia rebaudiana	Steviol (**24**) sclareol (**22**), 11*E*,13(16)-labdadiene-6α,7β,8α,14,15-pentol, 14-epi-11*E*,13(16)-labdadiene-6α,7β,8α,14,15-pentol, stevioside (**20**), dulcoside A **(20a)**, rebaudioside A (**25**), rebaudioside B (**25a**), rebaudioside C (=dulcoside B) (**25b**), isosteviol, steviolbioside, rebaudioside D (**25c**), rebaudioside E (**25d**), rebaudioside F (**25e**), dulcoside A (**25f**)	Food additive (sweetener) anticancerous, antihyperglycemic, antihypertensive, anti-inflammatory, antimicrobial, contraceptive, diminishing cramps, hot flashes, night sweats and pain Beneficial: for balancing, organoleptic
Stevia salicifolia		Antimicrobial, effective: in diminishing pain, cramps, night sweats, and hot flashes, Beneficial: for balancing hormone
Stevia samapatensis	2β-Hydroxy-7-labda-15-oic acid	
Stevia subpubescens	Subpubescensoside	
Stoechisoermum marginatum (algae)	5*R*,19-Diacetoxy-15,18-(*R*,*S*)-dihydrospata-13,16-diene	
Suegada multiflora	*ent*-16β,17-Dihydroxy-3-kauranone (=abbeokutone), *ent*-16-kaurene-3β,15β,18-triol, *ent*-3-oxo-16-kaurene-15β,18-diol, *ent*-kaurene-3β,15β-diol, helioscopinolide A, C, I	Antiallergic
Symphiopappus compressus	*ent*-2α-Hydroxy-3,13-kolavedien-15,16-olide	
Symphyopappus reticulatus	3,13-Clerodadiene-15,17-diol (=*ent*-3,13-kolavadiene-15,16-diene)	
Syzygium fluviatile	Acetyl incensole, cassipourol, cassipouryl formate, 4',5'-dehydrodiodictyonema	
Syzygium jambos	Phytol	Beverage (herbal tea) Antidiabetes, antioxidant
Tagetes gracilis	Isocimenone, 14,15-dihydro-bisocimenone	
Tawanaia cryptomeroides	Hinokiol	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Taxodium districhum	7β,12-Dihydroxy-8,12-abietadiene-11,14-dione (=taxoquinone, 7β-hydroxyroyleanone), 6α,11-dihydorxy-7,9,11(13)-abietatrien-11-one (=taxodone), 11-dihydorxy-7,9,11(13)-abietatrien-6,12-dione(=taxodione) 12-hydroxy-5,8,11,13-abietatetraen-7-one (=5,6-dehydrosugiol), sugiol, Δ^5-dehydrosugiol	Tumor inhibitor
Taxodium mucronatum	15-Pimarene-8β,19-diol	
Taxus baccata	2α,5α,7α,9α,10β,13α,-Hexaacetoxy-11-taxen-1β-ol (=1β-hydroxybaccatin I), 2α,4α,7α,9α,10β,13α-hexaacetoxy-5β,20-epoxy-11-taxen-1β-ol (baccatin IV), 4α,10β-diacetoxy-2α-benzoyloxy-5β,20-epoxy-1β,6β,13-trihydroxy-11-taxen-9-one (=baccatin III) (**53**), baccatin V-VII	Tumor inhibitor
Taxus brevifolia	Paclitaxel (=taxol) (**1**), 7-epitaxol,10-deacetyl-10-oxo-7-epitaxol	Tumor inhibitor
Taxus cuspidata	1β-Hydroxy-7β-acetoxytaxinine, 1β,3β-dihydroxytaxinine	Tumor inhibitor
Taxus mairei	1β,2α,7α,9α,10β,13α-Hexaacetoxy-4β,20-epoxy-11-taxen-5α-ol (=1β-acetoxy-5-deacetylbaccatin I), 1-dehydroxy-4-deacylbaccatin IV	Tumor inhibitor
Taxus sumatrana	Taxanes	Cytotoxic (Hepa59T/VGH: liver carcinoma, NCI: lung carcinoma; HeLa;DLD-1: colon cancer, Med: human medulloblastoma cell lines)
Taxus wallichiana	5,1α-Epoxy-1,2,3,7,10,13,19-heptahydroxy-11-taxen-9-one, 10-deacetyltaxol, cephalomannine (**52**), deacetylcephaloannine	Tumor inhibitor
Taxus yunnanensis	5,20-Epoxy-2,4,7,10,13-pentahydroxy-11-taxen-9-one (=1-dehydroxybaccatin III, 5,20-epoxy-11-taxen-2,4,7,9,10,13-heptaacetate (=1-acetylbaccatin IV)	Tumor inhibitor

Tedania ignis	Tedanol	COX-II inhibitory
Tetraclinis articulate	8,11,13-Abietatriene-3-oxo-12-ol (=hinokione), 8,11,13-totaratrien-13-ol (=totarol) (**180**)	
Tetraclinis sp.	8,11,13-Abietatriene-3β,13-diol	
Tetradenia riparia (=Iboza riparia) (ginger bush)	7α-Hydorxyroyleanone (=ibosol), 8(14),15-sandaracopimaradiene-7α,18-diol, 8-abieten-7β,13β-diol	Beverage (herbal tea) Antifungal, antileishmanial, antimicrobial, antispasmodic, antiviral For cough, diarrhea, dengue fever, headaches, malaria, toothache
Teucrium abutiloides	*ent*-4β,18:12,20:15,16:19, 20-Tetraepoxy-13(16),14-clerodadien-6β-ol (=teubutilin A, *ent*-4β,18:12,20:15,16-triteepoxy-13(16)14-clerodadien-6β,19,20-triacetate (=teubutilin B)	
Teucrium bicolor	*ent*-15,16-Epoxy-9-hydroxy-3,13(16),14-clerodatrien-19,6:20,12β-diolide (=*ent*-12α*H-epi*-teuscordonin)	
Teucrium botrys	*ent*-15,16-Epoxy-6α,12*R*, 18-trihydroxy-3,13(16), 14-clerodatrien-20,19-olide (=teubotrin), 15,16-epoxy-6α,18,19-trioxy-3,13(16),14-clerodatrien-20,12-olide (=tausalvin C)	
Teucrium buxifolium	*ent*-4β,18;15,16-Diepoxy-3α-hydroxy-6-oxo-19-acetoxy-12(16),14-clerodadien-20,12-olide (=19-acetylteulepicin)	
Teucrium canadense	Teuflin, teuevidin, isoteyflin	
Teucrium capitatum	*ent*-15,16-Epoxy-6α,7α, 18,19-tetrahydroxy-3,13(16),14-clerodatrien-20,12β-olide (=lonin)	Flowered aerial part: Beverage (herbal tea, liqueur "Terbert" and seasoning olives or soups)
Teucrium chamaedrys (Germander)	Teucrin A (**176**), dihydroteugin, isoteuflidin, teuflin, teuevidin, teuflidin, teuerun, 12(*S*)-15,16-epoxy-19-hydroxy-neocleroda-13(16),14-dien-18,6α:20, 12-diolide, teuchamaedrin, (12*S*)-15,16-epoxy-2β,6β-dihydroxy-neocleroda-13(16),14-dien-18,19:20, 12-diolide (=dihydroteugin), *ent*-4α,6α:15,16-diepoxy-2α-hydroxy-13(16),14-clerodadiene-18.19:20,12-olide (=chamaedroxide), *ent*-15,16-epoxy-2α,6α-dihydroxy-3,13(16),14-clerodatriene-18.19:20,12-olide (=teugin), *ent*-	Flower aerial part: Beverage (herbal tea, bitter alcoholic, bitters and liqueurs, flavored wine) Antibacterial, antioxidant, astringent, hepatotoxicity For abscesses, digestion, gout, respiratory disorders, infusion on the gums, wounds healing

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
	2β,3β:15,16-diepoxy-4α,6α-dihydroxy-13(16), 14-clerodadiene-18.19:20,12-olide (=teucrin F), *ent*-2β,3β:15,16-diepoxy-4α,6α-dihydroxy-3(16),14-clerodadiene-18,19:20,12α-olide (=teucrin G), *ent*-15,16-diepoxy-6α-hydroxy-3(16),14-clerodadiene-18,19:20,12α-olide (=teucrin H), *ent*-4β,19β:15,16-diepoxy-2α,6α-18-trihydroxy-13(16),14-clerodadien-20,12-olide (=teucroxide), *ent*-15,16-epoxy-19-*nor*-3,13(16),14-clerodatrien-18,6α:20,12-olide (=teuchamaedryn A), *ent*-15,16-epoxy-7β-hydroxy-19-nor-4,13(16),14-clerodatrien-18,6β:20,12-olide	
Teucrium divaricatum	*ent*-4α,6a:15,16-Diepoxy-13(16),14-clerodadiene-18,19:20,12-olide (=chamaedroxide)	
Teucrium eriocephalum	*ent*-4β,18:12,20:15,16-Triepoxy-7β-hydroxy-19,20-diacetoxy-13(16),14-clerodadien-6-one (=eriocephalin)	
Teucrium flavum	*ent*-19-Acetoxy-4β,18:12,20:15,16-triepoxy-6β,20β-dihydroxy-13(16),14-clerodadien-3-one (=teuflavin), *ent*-15,16-epoxy-18-hydroxy-19-*nor*-4,13(16),14-clerodatrien-20,12-olide-6β-*O*-(2-acetyl-1β-*D*-glucopyranoside (=teuflavoside), *ent*-15,16-epoxy-19-*nor*-3,13(16),14-clerodatriene-18,6α:20,12-olide (=teufin), tauflin(=C-10 epimer of teucvidin)	Flavor wines, bitters, liqueurs and a hop substitute For all kinds of rashes and skin problems
Teucrium fragile	*ent*-15,16-Diepoxy-2α,6α-dihydroxy-3,13(16),14-clerodadtrien-18,19:20,12-diolide-6-one (=teugin), 15,16-epoxy-2β,6β-dihydroxy-*neo*-cleroda-3,13(16),14-triene-18,19:20,12*S*-diolide	

Teucrium fruticans	*ent*-4β,18:15,16-Diepoxy-1-hydroxy-19-acetoxy-13(16),14-clerodadien-6-one (=isofruticolone), *ent*-19-acetoxy-4β,18:15,16-diepoxy-1β,8α-dihydroxy-13(16),14-clerodadien-6-one (=8β-hydroxyfruticolone), *ent*-15,16-diepoxy-18-hydroxy-19-*nor*-4,13(16), 14-clerodadien-20.12-olide-6β-*O*-(2-acetyl-β-*D*-glucopyranoside (=teuflavoside), fruticolone,7β-hydroxyfruticolone, 11-hydroxyfruticolone, deacetylfruticolone, 6-acetyl-10-hydroxy-teucjaponin B, (16*S*)-12,16-epoxy-11,14-dihydroxy-17(15 → 16),18(4 → 3)-diabeo-abieta-3,5,8,11,13-pentaene-2,7-dione (=teuvincenone E), taufruintin A	Antifeedant (*Spodoptera littoralis*)
Teucrium gnaphaloides (*T. polium* var. *gnaphalodes*)	*ent*-4β:18:15,16α-Diepoxy-6-oxo-19-acetoxy-13,16(14)-clerodadien-20,12β-olide (=19-acetylgnaphalin), *ent*-4β:18:15,16α-diepoxy-6-acetoxy-19-hydroxy-13,16(14)-clerodadien-20,12α-olide (=12-epiteupolin II), *ent*-4β:18:15,16α-diepoxy-6-oxo-19-hydroxy-13, 16(14)-clerodadien-20,12β-olide (=gnaphalin), *ent*-4β:18:12,20:15,16α-triepoxy-6-oxo-19,20-diacetoxy-13,16(14)-clerodadiene-(=gnaphalidin), 6β,18; 15,16-diepoxy-4α,6α,12*S*-trihydroxy-*neo*-clerodane-13(16),14-dien-20,19-olide (=teugnaphalodin)	
Teucrium hyrcanicum	*ent*-15,16-Epoxy-3α-hydroxy-19-nor-4,13(16), 14-clerodatriene-18α,6α:20,12-diolide (=teucrin H_1), *ent*-15,16-epoxy-6α-hydroxy-13(16),14-clerodadiene-18,19:20,12-diolide (=teucrin H_2), *ent*-4β,18:15,16α-Diepoxy-6-oxo-19-acetoxy-13,16(14)-clerodadien-20,12-diolide (=teucron H_3, =19-acetylgnaphalin), *ent*-15,16-epoxy-2β-hydroxy-19-nor-4,13(16), 14-clerodatriene-18,6a:20,12-diolide (=teucrin H_4)	

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Teucrium japonicum	*ent*-4b:18:15,16α-Diepoxy-6,19-dihydroxy-13,16(14)-clerodadien-20,12β-olide (=teucjaponin A), *ent*-4b:18:15,16α-diepoxy-6-acetyl-19-hydroxy-13,16(14)-clerodadien-20,12β-olide (=teupolin II), *ent*-4b:18:15,16α-diepoxy-6-thydroxy-19-acetoxy-13,16(14)-clerodadien-20,12β-olide (=teupolin I, =teucjaponin B), (12*S*)-19-*nor*-*neo*-cleroda-15α-hydroxy-4,13-diene-16,15; 18,6β; 20, 12-triolide (=teuponin)	Insect antifeedant
Teucrium kotschyanum	*ent*-15,16:18.19-Diepoxy-6α,18α-dihydroxy-13(16),14-clerodadien-20,12-olide (=teukotschyn), *ent*-10βH,15,16-epoxy-19-*nor*-3,13(16),14-clerodatriene-18,6a:20,12-diolide (=12-epiteucvidin), *ent*-10α*H*-15,16-epoxy-19-nor-3,13(16),14-clerodatriene-18,6α:20,12-diolide (=12-epieufin), *ent*-15,16-epoxy-19-*nor*-5(10),13(16),14-clerodatrien-18,6a:20,12-diolide (=isoteucrin H)	
Teucrium lamiifolium	*ent*-4b:18:15,16α-Diepoxy-6-acetoxy-19-hydroxy-13,16(14)-clerodadien-20,12-olide (=12-epiteupolin II), *ent*-15,16-epoxy-6α,12*R*, 18-trihydroxy-3,13(16),14-clerodatrien-20,19-olide 18,6α:20,12-diolide (=teulamifin), teuscordinon, teuflin, montanin C, 19-acetylgnaphalin.	
Teucrium lanigerum	Eriocephalin: 20-deacetyleriocephalin, [19-acetoxy 4α,18:15,16-diepoxy-7α-hydroxy-6-keto-*neo*-cleroda-13(16),14-diene-20*S*,12*S*-hemiacetal], isoeriocephalin: [19-acetoxy-4α,18:15,16-diepoxy-6α-hydroxy-7-keto-neocleroda-13(16), 14-diene-(20-acetyl)-20*S*, 12*S*-hemiacetal]	

Teucrium lepicephalum	*ent*-4β:18:15,16-Diepoxy-3,19-dihydroxy-6-oxo-3(16),14-clerodadien-20,12-olide (=teulepicin), *ent*-4β:18:15,16-diepoxy-3-hydroxy-6-oxo-19-acetoxy-3(16),14-clerodadien-20,12-olide (=19-acetylteulepicin), *ent*-6α,18:15,16-diepoxy-3α,4β,6β,12-tetrahydroxy-13(16),14-lerodadien-20,19-olide (=teulepicephin)	
Teucrium marum	9-Acetoxy-4α,18:15,16-diepoxy-2β,6β-dihydroxy-neocleroda-13(16),14-dien-20,12*S*-olide (=Teumarin)	Flowered aerial part: for making liqueur Whole plant: gallbladder and stomach problems, nervous complaints Root bark: hemorrhages Flowered aerial part: antibacterial, antimicrobial, antioxidant
Teucrium massiliense	*ent*-4β:18-Epoxy-6,19-dihydroxy-13-cleroden-16,15-olide (=deacetylajugarin II), ajugarin, deacetylajugarin, 4α,18:15,16-diepoxy-6α, 12*S*,19-trihydroxy-*neo*-cleroda-13 (16),14-diene (=teumassilin), 6α,19-diacetoxy-4α,18:15,16-diepoxy-12*S*-hydroxy-*neo*-cleroda-13(16),14-diene (=6,19-diacetylteumassilin), triacetylteumssilin, montanin C, teucjaponin A	Antibacterial
Teucrium micropodioides	*ent*-4β:18,12,20:15, 16:19,20-Tetraepoxy-13(16),14-clerodadine-3α,6α-diol (=teumicropin), 3-acetylteumicropin	
Teucrium montanum	*ent*-4β:18:15,16α-Diepoxy-6,19-diacetoxy-13,16(14)-clerodadien-20,12β-olide (=teupolin II), *ent*-4β:18:15,16α-diepoxy-6-hydroxy-19-acetoxy-13(16),14-clerodadien-20,12β-olide (=teupolin I, =teucjaponin B), *ent*-6,18:15,16-diepoxy-19-nor-4(18),5,13(16),14-clerodatrien-20,12-olide (=montanin A), *ent*-15,16-diepoxy-6β,18-dihydroxy-19-*nor*-4,13(16),14-clerodatrien-20,12-olide (=montanin B), *ent*-4β,19:15,16-diepoxy-6α,18-dihydroxy-13(16), 14-	Beverage (Tea, known as mountain germander) Analgesic, antidepressant, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, diuretic, stomachic

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
	clerodadien-20,12-olide (=montanin D), *ent*-15,16α-epoxy-4α,6α, 18,19-tetrahydroxy-13(16),14-clerodadien-20,12-olide (=montanin E), *ent*-4β:18:15,16α-diepoxy-3α-hydroxy-6β,19-diacetoxy-13(16)-cleroden-20,12β-olide (=montanin G), montanin C	
Teucrium pestalozzi	Teupestalin A, B, 20-oxoteuflavin, *ent*-4β:18:12,20b:15,16-triepoxy-3α-hydroxy-13(16),14-clerodadien-3,6,19-tetrol (=4,8-epoxyafricanin)	
Teucrium polium	Teulolin A (**177**), B, polivincins A, B, teulamifin B, montanin B, D, teupolin XII, teucrun P1, teucrin H_3, montanin B, 19-deacetylteuscorodol, teucroxide, teupolins I-V, teukamifin B, *ent*-4β:18:15,16α-diepoxy-6-acetyl-19-hydroxy-13,16(14)-clerodadien-20,12β-olide (=teupolin II), *ent*-4β:18:15,16α-diepoxy-6-hydroxy-19-acetoxy-13,16(14)-clerodadien-20,12β-olide (=teupolin I,=teucjaponin B), *ent*-12:20:15,16,19,20-triepoxy-4β,18-dihydroxy-13(16),14-clerodadien-6-one-6-one (=teupolin III), *ent*-6-acetoxy-4β,18:7,19:15,16-triepoxy-7α-hydroxy-13(16),14-clerodadien-20,12-olide (=isopocropolin), *ent*-4β,8:12:20:15,16-triepoxy-13(16),14-clerodadien-6-one (=teucrin P_1), teupolin V, *ent*-6-oxo-4β,18:7β,20: 15,16-triepoxy-12R,19-diacetoxy-13(16),14-clerodadien-20α-ol (=auropolin), teulamifin B, 15,16-epoxy-6β,12*S*,18-trihydroxy-neocleroda-3,13(16),14-trien-20,19-olide	Flowered aerial part: as Beverage (herbal tea), Leaves, flowered aerial part: Antibacterial, anticancer, antifungal, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, hypoglycemic, hypolipidemic For common cold, gastrointestinal disorders, indigestion, type 2 diabetes, rheumatism For abdominal pain, diabetes, indigestion Infusion: stomachic, tonic
Teucrium polium subsp. *vincentinum*	12,16*S*-Epoxy-6,11,14-trihydroxy-17(15→ 16)-abeo-3,8,11,13-abietateraene-3,7-dione (=teuvincenone A), 12,16*S*-epoxy-6,11,14-trihydroxy-17(15→16)-abeo-3,8,11,13-abietateraene-7-dione, teuvincenone B-D, teupolins	Flowered aerial part: beverage (herbal tea) Cytotoxic (Hep-G2: hepatoblastoma cancer cell line)

Teucrium pyrenaicum	*ent*-4,18:15,16-Diepoxy-3,6,19-triacetoxy-13(16), 14-clerodadien-20,12-olide (=teupyreinin), *ent*-4β,18:12,20:15, 16:19,20-tetraepoxy-3-acetoxy-13(16),14-clerodadien-6-one (=teupyrenone), *ent*-3α,12*S*-diacetoxy-4,18:15,16:19,20-triepoxy-20*R*-hydroxy-13(16),14-clerodadien-6-one (=teupyrin A), *ent*-4β,18;12β*H*,20β: 15,16-triepoxy-3α,6β, 19,20-tetracetoxy-13(16), 14-clerodadiene (=teupyreinidin), 6α-acetoxy-4α,18;15,16-diepoxy-3β,12ξ,19-trihydroxy-neocleroda-13(16),14-diene (teupyrin B), teupureinidin, teucrin P1, 3β-acetoxy-teupyreinin, teumassilin	Flowered aerial part: beverage (herbal tea)
Teucrium salviastrum	*ent*-15,16:18,19-Diepoxy-2α,18α-dihydroxy-6-oxo-3,13 (16),14-clelordadien-20,12-olide (=teusalvin A), *ent*-15,16:18,19-diepoxy-18α-hydroxy-2,6-dioxo-13(16),14-clelordadien-20,12-olideteusalvin B, *ent*-15,16-epoxy-6α,18,19-trihydroxy-3,13(16),14-clelordatrien-20,12-olide (=teusalvin C), *ent*-15,16-epoxy-2α,6α,18,19-tetrahydroxy-3,13(16),14-clelordatrien-20,12β*H*-olide (=teusalvin D), *ent*-15,16-epoxy-2α,6α, 12*R*-tetrahydroxy-3, 13(16),14-clelordatrien-20,19-olide-olide (=teusalvin E), *ent*-2β,19:15,16-diepoxy-6α,18-dihydroxy-3,13(16),14-clelordatrien-20,12-olide (=teusalvin F)	Antioxidant, anti-inflammatory
Teucrium scordium	*ent*-15,16-Epoxy-6β-hydroxy-1,13(16),14-clerodatriene-18:19:20,12-diolide (=2,3-dehydroteucrin E), *ent*-15,16-epoxy-2α-hydroxy-6-oxo-3,13(16), 14-clerodatriene-18:19:20,12-diolide (=hydroxyteuscordinone), *ent*-15,16-epoxy-5-hydroxy-3,13(16),14-clerodatriene-18:19:20, 12-diolide (=*ent*-6-hydroxyteuscordin), *ent*-15,16-epoxy-5-hydroxy-6-oxo-3,13(16), 14-clerodatriene-18:19:20,12-diolide (=teuscordinone), *ent*-15,16-epoxy-5,19-dihydroxy-2-oxo-3,13(16),14-clerodatriene-19,6:20,12-diolide (=2-keto-19-hydroxy-teuscordin), (-)-6β-hydroxyteuscordin, 6-acetylteucjaponin B	Anthelmintic, antifungal, antiseptic, diaphoretic, skin, tonic

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Teucrium scorodonia	*ent*-15,16-Epoxy-6α-hydroxy-19-acetoxy-3,13(16),14-clerodatrien-18-al-20,12β-diolide, *ent*-15,16:18.19-diepoxy-6-oxo-18α-hydroxy-13(16),14-clerodadien-20,12-olide (=teukotschyn), *ent*-15,16-epoxy-9-hydroxy-3,13(16),14-clerodatrien-19,6:20,12α-diolide (=teuscorodonin), *ent*-15,16-epoxy-6α,18-dihydroxy-19-acetoxy-3,13(16),14-clerodatrien-20,12β-diolide (=teuscoradol), *ent*-15,16-epoxy19-*nor*-4,6,13 (16),14-cleroda-tetraenehydroxy-18:6:20, 12-diolide (=teuscorolide), *ent*-15,16-epoxy-2α-hydroxy-19-nor-4,6, 13 (16),14-clerodatetraene-18:6:20,12-diolide (=2-hydroxyteuscorolide), 19-acetoxy-6α-hydroxy-4α, 18: 15,16-diepoxy-neoclerodane-13(16),14-dien-20,12*S*-olide (=eupolin I), 19-acetoxy-6β-hydroxy-15,16-epoxy-*neo*-clerodane-3,13 (16),14-trien-20,12*S*-olid-18-al (teuscorodal)	Beverage (herbal tea) Leaves and flowers: a hop substitute for flavoring beer Astringent, carminative, diaphoretic, diuretic, emmenagogue, tonic, vulnerary
Teucrium spinosum	*ent*-15,16-Epoxy-4β,19-dihydorxy-13(16),14-clerodadien-19,6α:20,12-diolide, teuspinin, 19-acetylteuspinin	
Teucrium viscidum	*ent*-15,16-Epoxy-19-*nor*-3,13(16),14-clerodatrien-18,6a:20,12-diolide (=teucvidin), teucvisins A-E, teucvin, teuflin, kinalborin C, teuspinin, 19-acetyl-teuspinin, isoteuflin, 2-deoxychamaedroxide, teuscordal, 6α-acetoxyteuscordin, teuvisone, biteuvisone A, B	Acetylcholinesterase inhibitory, antidiabetes, antifeedant, anti-inflammatory antimicrobial Chinese folk medicine Hematemesis, pulmonary abscesses, traumatic injuries, and bites from rabid dogs or venomous snakes
Teucrium webbianum	*ent*-15,16-Epoxy-2α-hydroxy-19-*nor*-3,13(16), 14-clerodatriene-18,6:20, 12-diolide (=*ent*-2α-hydroxyteucvidin), teuflidin, teucrin A, *ent*-2α-hydroxyteucvidin	
Thuja occidentalis	Beyerene, dehydro-abietane, neothujic acids III, IV, (+)-7-oxo-13-*epi*-pimara-14,15-dien-18-oic acid, (+)-7-oxo-13-*epi*-pimara-8,15-dien-18-oic acid, (+)-isopimaric acid	Food: Pith of young shoots Leafy branchlets: beverage (herbal tea) Anthelmintic, anti-inflammatory, antiseptic, antiviral, benzo(*a*)pyrene-protein binding inhibitor

Thuja plicata	18-Nor-8(14),15-iso-pimaradien-4-ol, 19-nor-8(14),15-iso-pimara-dien-4-ol,*syn*-stemod-13(17)-ene, sandaracopimaradiene, levopimaradiene, rimuene, beyerene, beyerene-1β-ol, kaur-15-ene, (*E*)-totarol, totaradiol, ferrugiol	Food: Inner bark used as a thickening in soups etc. or mixed with cereals when making bread, chewing gum Leaf: for various internal pains Stem and root: for colds Seeds and twigs: for fevers Antimicrobial
Thuja standishii	15-Isopimaren-8β-ol, 15,16-bisnor-13-oxolabda-8(17),11*E*-dien-19-oic acid, (E)-communic acid (**3**), 15-oxolabda-8(17),11*Z*, 13*E*-trien-19-oic acid, 15-oxolabda-8(17),11*Z*,13*Z*-trien-19-oic acid, 12-oxo-11-nor-drim-8-en-14-oic acid, 15-oxolabda-8(17),13*Z*-dien-19-oic acid, 15-nor-14-oxolabda-8(17),12*E*-dien-19-oic acid, ferruginol, sugiol, isocupressic acid, sandaracopimaric acid, standishinal (=8-formyl-6(7→11)*abeo*abietane)	Antitumor, aromatase, and EBV-EA inhibitory
Thujopsis dolabrata	16-Hydroxyferruginol (**178**), (15*R*)-12,16-epoxy-8,11,13-abietatriene (**179**), totarol (**180**), 7α-hydroxytotarol (**181**), 7β-hydroxytotarol (**182**), rimuene, rosa-5,15-diene, 13-*epi*-dolabradiene, hibaene, dolabradiene, isoagatholal-15-*O*-β-*D*-xylopyranoside	Soap flavor, aroma retaining agents Antibacterial, antifungal, anti-inflammatory, antiproliferative, antiviral For hypertension, jaundice rheumatoid, liver and kidney disorders
Thymus pulegioides (leaf thyme)		Flowered aerial part: beverage (herbal tea, liqueur: ratafia) liqueur (ratafia), for seasoning stews For sedation, blood purification, renal stones, bronchitis, stomach spasms, asthma, diarrhea, flatulence, fever, airway decongestant, gastrointestinal disinfectant, depurative, disinfect wounds, ulcers, cold, digestive
Thymus serphyllum (wild thyme)		Beverage (herbal tea) Antibacterial, antidiarrheal, antimalarial, antioxidant, antiproliferative, antihypertensive, antiseptic, anthelmintic, antitussive, bronchitis, expectorant, sedative, spasmolytic, tonic For eczema, menstrual disorders, rheumatism, wounds

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Thymus vulgaris	Carnosol (**16**), epirosmanol (**183**), methyl carnosate, carsonic acid (**169**)	Flowered aerial part: beverage (herbal tea), spice Analgesic, anthelmintic, anti-bronchiolitis, anticatarrhal, antifungal, antihypertensive, anti-inflammatory, antimicrobial, antioxidant, antitussive, antispasmodic, carminative, diuretic, expectorant For acne, gastrointestinal, stomatitis
Thymus zygis (Spanish thyme)		Aerial part (flowered): Beverage (herbal tea), spice Antibacterial, anti-inflammatory, antioxidant
Tinomiscium philippinense	Tinophyllone	Foods, beverage (Herbal tea), cosmetics, pharmaceutics Antimicrobial, antitumor, insecticide, mosquito repellent
Tinospora cordifolia	Epoxyclerodanes	Gastroprotective
Tinospora cordifolia (=*T. sinensis*)	2β,3β:15,16-Diepoxy-4α,6α-dihydroxy-2,13(16),14-clerodatriene-17,12:18,1β-diolide, 15,16-epoxy-4α,8β-dihydroxy-2,13(16),14-clerodatrien-17,12:18,1β-diolide, *ent*-15,16-diepoxy-4α-hydroxy-2,13(16),14-clerodatriene-17,12:18,1β-diolide (=columbin), tinosporafuranolactone, 15,16-epxy-6α-hydroxy-19-nor-4,13(16),14-clerodatrien-17,12-olide-18-oic acid *O*-β-*D*-glucopyranosyl ester, 15,16-epoxy-4α-hydroxy-18-nor-1-oxo-2,13(16),14-clerodatrien-17,12-olide, cordifoliosides A-E, tinocordioside, cordioside	Stem: used as Ayurveda, folk medicine Antiallergic, antiarthritic antidiabetic, anti-inflammatory, antileprotic, antimalarial, antioxidant, antiperiodic, antispasmodic, antistress, hepatoprotective, immunomodulatory
Tinospora malabarica (=*Tinospora sinensis*)	10α-Hydroxycolumbin, malabarolide, 1-acetyltinosinenoside D, E, tinosinenoside D-F, K	Stem: used as Ayurveda, Folk medicine, Traditional Chinese Medicine (TCM) Antiarthritic, antidiabetes, anti-inflammatory, immunomodulatory

Tinospora tuberculate (=*T. crispa, T. rumphi)*	15,16-Epoxy-3β,4α-dihydroxy-13(16),14-clerodadiene-17,12:18,6-diolide (=borapetol A), borepetol B, borapetosides A-F, columbin, rumphioside A-F, tinotufolin A, B	Stem: used as a folk medicine Antidiabetes, anti-inflammatory, antimicrobial, antioxidant, cardiotonic
Torreya nucifera	8(17),13-Labdadiene-15,18-diol, torreferol, 8-hydroxyferruginol, 6-hydroxydehydroabienol, hinokiol, 4-*epi*-agathadiol, 18-hydroxy-13-epi-manool 18-methyl ester, ferruginol, 18-dimethoxyferruginol	Food, seed: edible oil Anthelmintic, antioxidant
Trachylobium verrucosum (=*Hymenaea verrucosa*)	13*S*,8(17)-Labden-15-oic acid	
Trigonastemon heterophylla	Trigonostemome, trigonochinene B	Antimicrobial
Triticum aestivum	Gibberellin A_{60}, gibberellin A_{61}, gibberellin A_{62}	Foods, beverage (herbal tea) Anticancer, anti-inflammatory For childhood asthma, preventing gallstones, post-menopausal symptoms, chronic inflammation
Trogopterus xanthipes	Manool **(34)**, 12*R*,13*R*-8,12-epoxy-14-labden-13-ol, pomiferin A, B, 11,13-abitatrien-7α,18-diol, 7-oxocallitrisic acid	Antithrombotic
Trupterygium wilfordii	triptolide, tripdiolide, triptobenzenes A-G	Root (also known as thunder god vine) Anti-inflammatory, antirheumatism, cartilage protective immune suppressive
Turbina corymbosa	6α,16β,17-kauranetriol	Seed: psychedelic drug
Turraeanthus africanus	Terraeanin	Antimicrobial
Udotea flabellum	Udoteatrial, udoteal, udoteafuran	Antimicrobial, antitumoral
Udotea petiolata (=*Flabellia petiolata*)	Udoteal	Induced feeding avoidance in the herbivorous fish (*Eupomacentrus leucostictus*)
	Udoteal B	Antibiotic, cell division inhibitory of fertilized sea urchin egg, toxicity against herbivorous damselfish

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Ulva lactuca	Labda-14-ene-3α,8α-diol, labda-4-ene-8-hydroxy-3-one	Food Anticancer, antimicrobial, antioxidant
Vellozia candida (=*Vellozia maritima, Vellozia tertia*)	5α,10α-Epoxy-7,11-dihydroxy-7,15-rosadien-6-one	
Vellozia piresiana	8β-Hydorxy-15-isopimaren-3-one (=epoxyvellozin), compactone, 7β,8β-dihydroxy-3-oxopimar-15-ene, 8β-hydroxy-3-oxopimar-15-ene, 8,11,13-cleistathatrien-3,7-dione, 1,8,11,13-cleistanthatetraen-3,7-dione	
Viburnum odoratissimum	Vivsonins A-C, E, F	Fruit
Vicia faba	Gibberellin A_{53} (**175**)	Food Antioxidant, bones strengthen, diuretic For anemia, lithotriptic
Viguiera arenaria	Kauranes, *ent*-Pimara-8(14),15-dien-19-oic acid, its sodium salt, *ent*-8(14),15-pimaradien-3β-ol, 3β-acetoxy-*ent*-15-8β,19-diol, *ent*-3β-acetoxy-8(14),15-pimaradiene	Antimicrobial, vascular contractility inhibitory
Viguiera robusta	Kauranes, pimaranes	Vascular contractility inhibitory
Vitex cauliflora	3-Oxo-15,17,18-triacetoxylabda-7,13*E*-diene	Anti-*Plasmodium falciparum*
Vitex negundo	Negundoins A-G	Antioxidant
Vitex rotundifolia	15,16-Diepoxy-14-labden-6β-ol, (*rel*-5*S*,6*R*,8*R*,9*R*,10*S*)-6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide, (*rel*-*S*,6*S*,8*R*,9*R*,10*S*)-6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide, (*rel*-*S*,6*R*,8*R*,9*R*,10*S*)-6-acetoxy-9-hydroxy-15-methoxy-13(14)-labden-16,15-olide, (*rel*-*S*,6*R*,8*R*,9*R*,10*S*,13*S*,16*S*)-6-Acetoxy-9,13-epoxy-16-methoxylabdan-15,16-olide, (*rel*-5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,16*S*)-6-Acetoxy-9,13-epoxy-16-methoxylabdan-15,16-olide, (*rel*-*S*,6*R*,8*R*,9*R*,10*S*,13*S*)-6-Acetoxy-9,13-epoxy-15-	Fruits “Viticis Fructus”: colds, eye pain, headaches, migraine Edible leaves: relieve illnesses such as wella (burning sensation), and nalulu (dull headache, dull pain in stomach, queasy) Antihyperlipidemia, antiplasmodial

	methoxylabdan-16,15-olide, (*rel-S*,6*R*,8*R*,9*R*,10*S*,13*R*)-6-acetoxy-9,13-epoxy-15-methoxylabdan-16,15-olide, (*rel-*5*S*,8*R*,9*R*,10*S*,13*S*, 15*S*,16*R*)-9,13;15,16-Diepoxy-15,16-dimethoxylabdane, (*rel-*5*S*,8*R*,9*R*,10*S*,13*S*,15*R*,16*S*)-9,13;15,16-diepoxy-15,16-dimethoxylabdane, vitexilactone, viterofolins A-H	
Vittadinia gracilis (=Vittadinia cuneate, Eurybiopsis gracilis)	*ent*-12α,20α:15,16-diepoxy-3,13(16),14-clerodatriene-17,20:18,19-diolide	Eaten regularly but not preferred by livestocks and wild animals
Xenia membranacea	Branacenal, 7,8:17,18-diepoxy-1(17),6(20),13-xeniatetraene-11,12,18-triol (=bisdioxyhavannahin)	
Xenia novaebrittanniae	Novaxenicins B, xenioide I	Antimicrobial
Xenia obscuronata	4(18),8(19)-xeniaphylladiene-5,14,15-triol (=xeniaphyllantriol), obscuronatin	
Xestospongia vanilla	xestenone	
Xylopia aethiopica	*ent*-15α-Acetoxy-kaur-16-en-18-oic acid (= xylopic acid) (**184**), 2-oxo-kolavenic acid (**185**)	Fruit: coffee drink, soups Biliousness and febrile pains, bronchitis, dysenteric Steeped bark in palm-wine: asthma, bronchitis, biliousness and febrile pains, dysenteric, rheumatism, stomachaches Macerate leaves in palm-wine: a locally popular intoxicating drink Analgesic, antiallodynic, antibacterial, antifungal, antihyperalgesic, condiments, cytotoxic (leukemia, pancreatic cells) For colic pain, headache, neuralgia, rheumatism
Xylopia aromatica	*ent*-13,16-Dihydroxy-8(17),14-labdadien-18-oic acid, *ent*-trachyloban-3β-ol, *ent*-atisan-16α,18-diol, *ent*-atisan-16α-ol-18-oic acid	Fruit: febrifuge Seeds: Flavor cooked foods, poisonous, and kill rats Shoots and stem-bark: aromatic and tonic Flowers: carminative tonic Beverage (herbal tea): strong diuretic for swellings of the legs

(continued)

Table 1 (continued)

Dietary plant and mushrooms names	Diterpenoids and related compounds	Applications, benefits, effects
Zingiber mioga	Galanal A (**28**), B, 8β,17-epoxylabd-12*E*-ene-15,16-dial (=miogadial) (**27**), mioganal, labd-12*E*-ene-15,16-(8β),17-trial (=miogatrial), geranyllinalool	Food (young flower shoot), food additives Anticancer, antihypertension, anti-inflammatory, antiviral, platelet aggregation, and 5-lipoxygenase inhibitory For abnormal and irregular digestion, blood flow improvement, fever, hematemesis, hypotension, menstruation, skin health, TR, PA1 activation
Zingiber officinale	Galanolactone (**26**)	Food, vegetable, food additives, spice and beverage (herbal tea) Antagonist at 5-HT3
Ziziphora clinopodioides (wild basil, wild calamint)	Phytol	Spice, vegetable, beverage (herbal tea) Antibacterial, anti-inflammatory, antioxidant, antiseptic carminative, expectorant, sedative For migraine, depression, fever, gastrodynia, heart disorders, vomiting
Ziziphora tenuior	Manool (**34**)	Beverage (herbal tea) Antidiarrheal, antifungal, antimicrobial, antioxidant, antiseptic, expectorant, febrifuge, pectoral effects For bladder stones, cough, diarrhea, dysentery, fever, gut inflammatory, painful menstruation, stomach tonic, uterus infection, wound healing
Zealandia guidonia	Cinnamoyloxy-6β-hydroxyzuelanin, zuelaguidins E, G	Anticancer For amenorrhea, emetic

skin aging by suppressing UVB-induced MMP-1 expression. (*E*)-Communic acid (**3**) shows considerable cytotoxicity against four human cancer cell lines in vitro. It also shows good antibacterial activity against *Mycobacterium aurum.*

17.2.2 *Ajuga* [Lamiaceae (=Labiate)]

The Lamiaceae is widespread family of about 220 genera comprising 400 species distributed in the most of the world. More than 400 species *Ajuga* are cited in the Index Kew-ensis. *Ajuga* species are rich sources of clerodanes and neoclerodanes. The presence of clerodane diterpenoids have been reported in less than 20% of the above mentioned number of the species. Many clerodanes and neoclerodanes isolated from the *Ajuga* show antifungal, antimicrobial, cytotoxic, hypotensive, hypoglycemic, and insect antifeedant activities (Coll and Tandron 2008).

17.2.3 *Annona* (Annonaceae)

The Annonaceae is a very large family, occurring about 120 genera and more than 200 species. The family is an economically important source of edible fruits and oils. Seed oils of some *Annona* species are used as edible oils and cosmetics such as soap products. Flowers of *Annona* are also used in perfumery and used as folk medicines for cancer prevention. *A. squamosa* (sugar apple) which has been used as snack or dessert indicates the presence of antiviral, anti-inflammatory, antidiabetic, analgesic, and antioxidant compounds. Its bark and fruits show antidiarrheal activity and hematinic, cooling, sedative, stimulant, expectorant, maturant tonic, and antiviral properties, respectively (Vanitha et al. 2011; Wu et al. 1996). Among 14 kauranes isolated from this species, 16β,17-dihydroxy-*ent*-kauran-19-oic acid (**4**) indicated significant activity against HIV-replication in H9-lymphocyte cells (Wu et al. 1996). *ent*-11α-Hydroxy-16-kauren-15-one (**5**) was found to show apoptosis in human promyelocytic leukemia HL-60 cells. 4α-Hydroxy-17,19-dinor-*ent*-kauren-16-one, 4β-hydroxy-16β*H*-18-nor-*ent*-kauren-17-oic acid, and their 4β,17-dihydroxy-6α-acetoxy-nor-*ent*-kaurene and two more kaurene derivatives showed cytotoxicity against SMMC-7721 and HepG2 cancer cell lines (Chen et al. 2020).

17.2.4 *Baccharis* (Asteraceae)

The *Baccharis* is a genus of very large plants, with 400–500 species, belonging to the Asteraceae which distributed mainly in the Neotropical regions, South America, and Mexico. Many of the *Baccharis* species have been used as traditional medicines for the treatment of pain, wounds, ulcers, gastrointestinal disorders, as spasmolytic, diuretic, and analgesics, and in the treatment of high fever, diabetes, and bacterial and fungal infections. *Baccharis* flowers are rich in nectar and several species are

good honey plants. Some *Baccharis* species have been used as beverages in the form of decoction and infusion of the whole plants.

The major diterpenoids in the *Baccharis* are the neoclerodanes. In addition, kaurane and labdanes have also been found in this genus. Labda-7,13*E*-dien-3β,15-diol (**6**) possesses antifungal activity against a few fungi, such as *Trichophyton mentagrophytes* and *T. rubrum*. A unique kaurene, *ent*-3,19-disuccinylkaur-16-ene from *B. rufescens*, shows anti-inflammatory activity (Abad and Bermejo 2007).

17.2.5 *Caesalpinia* (Fabaceae)

The *Caesalpinia* is a genus of flowering plants belonging to the Fabaceae family. It contains tropical or subtropical woody plants and about 160 species have been known. The genus mainly produces the cassane type diterpenoids which indicate potent cytotoxicity against human cancer cell lines.

17.2.6 *Callicarpa* (Verbenaceae or Lamiaceae)

The genus *Callicarpa* have more than 40 species in China and Southern Asia, two representative of which are native or naturalized to the southeastern United States, namely *C. americana* and *C. dichotoma* (=*C. purpurea*). Many of the species have been ethnomedically used in the treatment of cancer, hepatitis, rheumatism, fever headache, indigestion, and the other ailments. Jones and Kinghorn (2008) reported the review article of *Callicarpa* constituents with biological active natural products including diterpenoids.

17.2.7 *Casearia* (Salicaceae)

The genus *Casearia* grown in South America comprises about 180 species most of which contain clerodane and kaurene diterpenoids with significant bio- and pharmacological activity, such as antifungal, antileishmanial, antiplasmodial, antitumor, trypanocidal, and antimalarial activity (Vieira-Junior et al. 2011).

17.2.8 *Cistus* (Cistaceae)

The *Cistus* is a genus of flowering plants in the rockrose family Cistaceae, containing about 20 species. They are perennial shrubs found on dry or rocky soils throughout the Mediterranean region, from Morocco and Portugal through to the Middle East, and also on the Canary Islands. Many labdanes possessing antimicrobial and cytotoxic activity, including manoyl oxide, have been found in this genus.

17.2.9 *Clerodendrum* (Verbenaceae)

The *Clerodendrum* is a genus of about 400 species in the family of the Verbenaceae which mainly grows in the tropic and warm temperature zone including southern Asia and Africa. It is currently classified in the subfamily Ajugoideae, being one of several genera transferred from Verbenaceae to Lamiaceae in the 1990s, based on phylogenetic analysis of morphological and molecular data. The genus is native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and southern Asia. The type species for the genus is *Clerodendrum infortunatum*. It is native to Sri Lanka and the Andaman Islands. The leaf aqueous extract of *C. glandulosum* has been used by the North-East Indian to alleviate symptoms of diabetes, obesity, and hypertension. Many insect antifeedant diterpenoids are distributed in this genus.

17.2.10 *Coffea* (Rubiaceae)

Coffee and tea are the two most important and consumed beverages in the world. About 80% of people in the world population take coffee marketed in the blended form of *Coffea arabica* and *C. canephora* (Novaes 2018).

Brazil, Colombia, Costa Rica, Elsalbador, Ethiopia, Guatemala, Honduras, Indonesia, Venezuela, and Vietnam are coffee export countries, and each country consumes 6.02 kg/one person/year, 1.91. 2.66. 2.59, 2.27, 1.29, 2.51. 0.099, 3.21, and 1.31, respectively. On the other hand, EU, Japan, Norway, Russia, Switzerland, Tunisia, Turkey, and the United States are coffee import countries and each county consumes 4.90 kg/one person/year, 3.54, 8.59, 1.70, 7.56, 2.48, 0.74, and 4.42, respectively. The presence of diterpenoids in green tea, English and Oolong tea is negligible while coffee beans contain pentacyclic diterpenoids which have not yet been isolated from or detected in the other foods, fruits, seeds, and vegetables. Since 30 years, the chemical and pharmaceutical studies of coffees were carried out from view point of food and beverage sciences.

Several observational studies indicate that coffee drinkers have a much lower risk of type 2 diabetes, a serious condition that affects millions of people in the world; anti-Alzheimer disorder, which is a leading cause of dementia, anti-Parkinson's disorder, the second most common neurodegenerative disease; and lower risk of liver and colorectal cancers. One of the most important discoveries of secondary metabolites from the coffees is the presence of the specific diterpenoids, cafestol (**7**), 16-*O*-methylcafestol (**8**), and kahweol (**9**) as the major components.

Arabic and Robusta coffee contain cafestol. 16-*O*-Methylcafestol has been found only in Robusta coffee. Arabica coffee also contains kahweol as predominant component; however, Robusta coffee produces kahweol as very minor diterpene. Filtered coffee does not contain cafestol or kahweol, because they are retained in filter paper. Instant coffee also does not contain diterpenoids. Diterpenoids in coffee beans' oil are present in free form. They are esterified with fatty acid, mainly palmitic acid, and linoleic oleanolic, stearic, arachidonic, and behenoic acids as major constituents. Up to

now, 100 *ent*-kaurane diterpenoids have been found in coffee. There are different kinds of diterpenoids produced during the process of fruit development and green bean roasting, although cafestol and kahweol are the main skeletons of coffee diterpenoids. Hu et al. (2020) reported the *ent*-kaurane diterpenoids including a lactam ring in roasted beans of Arabica coffee which inhibit α-glucosidase. Higher risk of heart disease has been known by up-taking of diterpenoids rich coffee. Although cafestol included unfiltered coffee, like Scandinavian-type decocted coffee, French-press cafeteria and Turkish coffee raise plasma triglycerol and LDL-cholesterol concentration in human in vitro and in vivo experimental results (Urgert and Katan 1997; Roos et al. 2001). Cafestol and kahweol shows pharmacological effects such as hepatoprotective, anti-inflammatory, antidiabetic, antiosteoclastogenesis, anticarcinogenic, and colon cancer inhibitory activities (Ren et al. 2019; Roos et al. 1997; Gross et al. 1997; Kurzrock and Speer 2001; Novaes 2018; Higdon and Frei 2007).

Kahweol inhibits COX-II expression, MCP-1 secretion, and NF-kB/STAT-1 pathway. In Japan population, habitual coffee drinking may be associated with reduced risk of hepatocellular carcinoma (HCC) (Inoue et al. 2005). Trinh et al. (2010) reported that decaffeinated coffee provided neuroprotection in *Drosophila* models of Parkinson's disease. Cafestol and its related diterpenes demonstrate their potential as multiple functional food and multiple target alternative medical drugs.

17.2.11 *Coleus* (Lamiaceae)

The *Coleus* grows in tropical to subtropical region. There are about 150 species and more than 500 varieties of the genus in cultivation all over the world. *Coleus eluteria* bark is used to flavor of the liqueur Campari and Vermouth.

One of the most important species of *Coleus* is *C. forskohlii* (=*Plectranthus barbatus*). The root of this plant has been used to treat heart disorders such as high blood pressure and chest pain (angina), as well as respiratory disorders such as asthma in Hindu and Ayurvedic traditional medicine from ancient times. Since its major labdane diterpene epoxide, forskolin (**10**) was found to have positive inotropic, antihypertensive, and adenylate cyclase-stimulating activities, and it has ever aroused a great deal of scientific interest, and a number of 8,13-epoxy-labd-14-en-11-one diterpenes have been obtained. Forskolin is also used to treat allergies, eczema and psoriasis, obesity, painful menstrual periods, irritable bowel syndrome (IBS), urinary tract and bladder infections, advanced cancer, blood clots, insomnia, and convulsions. Healthcare providers sometimes give forskolin intravenously for heart failure. Forskolin drops are used in the eyes to treat glaucoma.

17.2.12 *Conyza* (Asteraceae)

The *Conyza* (horseweed) has 150 species belonging to the Asteraceae family. It grows in tropical and subtropical regions. The representative diterpenes of this genus are clerodanes.

17.2.13 *Croton* (Euphorbiacae)

The genus *Croton* belonging to the Euphorbiacae family, one of the largest genera of higher plants, comprises more than 1300 species which distribute in tropical and subtropical areas of the world. Many species in this genus contain the secondary metabolites which show antibacterial, antidiabetic, antidiarrheal, antifungal, anti-inflammatory, antimalarial, antipyretic, antihypertensive, antispasmodic, antiulcer antiviral, insecticides, vermifuges, diuretic, cytotoxic, gastrointestinal disease, etc.

Diterpenoids are very rich in *Croton*, corresponding to clerodanes 800 of which have been isolated and their structures elucidated. In addition, it contains cembranes, halimanes, kauranes, labdanes, trachylobanes, sarcopetalanes type diterpenoids as well as phorbol esters (Xu et al. 2018; Palmeira et al. 2006; Salatino et al. 2007).

17.2.14 *Copaifera* (Fabaceae)

This genus belongs to the family the Fabaceae has about 70 species distributed in the central and South America especially in Brazil. Copaiba oil was approved in the United States as a food additive and is applied to a small amount as a flavoring agent in foods and beverage. The oleoresin or bark decoction has been used as an anti-inflammatory and contraceptive by native Brazilian people. Copaiba has a number of ethnophamacological indications for the treatment of respiratory ailments such as bronchitis, strep throat, hemoptysis, pneumonias and sinusitis, and skin and mucosa diseases, such as dermatitis, eczema, psoriasis, wounds, ulcers and lesions of the uterus, leishmaniosis and leucorrhea, anemia, headaches, etc. The oil is also used for syphilis, bronchitis, cough, rheumatism, aphrodisiac, stimulant, antiseptic, anti-herpetic, anthelminthic, anticancer, antiparalytic, and antitumor properties. Tea from the seeds of copaiba is also used as a purgative and for treatment of asthma. Copaiba oil is now used in the cosmetic industry as a fixative for perfumes and perfuming soaps and shampoo. *Copaifera* oleoresins contain not only sesquiterpenoids but also much amount of diterpenoids especially, clerodanes, kauranes, and labdanes among which copalic and kaurenoic acids indicate antibacterial, anti-inflammatory, antileishmanial, antitrypanosomal, cytotoxic, and wound healing properties (Trindade et al. 2018; Arruda et al. 2019; Vargas et al. 2015; Leandro et al. 2012; Custodio and Veiga 2012).

17.2.15 *Eremophila* (Scrophulariaceae)

The *Eremophila* is a genus of more than 260 species of plants in the figwort family, Scrophulariaceae all of which are endemic to mainland Australia. The *Eremophila* are widespread in the arid areas of Australia, especially Western Australia and range in size from low-growing shrubs to small trees. The major diterpenoids found in this genus are naturally rare serrulatanes possessing α-glucosidase inhibitory activity.

17.2.16 *Euphorbia* (Euphorbiacae)

The *Euphorbia* is a genus of plants in the Euphorbiacae family (7500 species and 275 genus) and there are 2100 species. It is one of the most diverse groups of flowering plants on the Earth. A great number of the species produce many highly oxidized different type of diterpenoids including abietanes, atisanes, casbanes, daphnane, euphanes, ingenanes, jatrophanes, kaurenes and lathlanes, myrsinane, rhamnopholanes, and tigliane. Their contents are very high in some species and their bioactivity is also very diverse. Since the discovery of anti-HIV activity in the early 1990s, macrocyclic diterpenoids in all plant groups have attracted many phytochemists and pharmacologists, and a number of papers concerning anti-HIV potential through PKC activation have been published. Not only such antiviral phytochemicals but also a number of diterpenoids found in this genus show potent cytotoxic activity against several human cancer cell lines, antimicrobial and antifungal activities.

17.2.17 *Ginkgo biloba* (Ginkgoaceae)

Every year about 8000 t of dried leaves of *Ginkgo biloba* are produced and widely sold as phytomedicine in Europe and as dietary supplement as beverage (tea). Now, about 50 millions of *G. biloba* tree are grown in Europe, China, France, the United States (South Carolina), and Japan. In Japan *G. biloba* seed is used as food and planted especially at roadside and as fire prevention tree. The acetone-water extract from the dried leaves contains structurally complex diterpene trilactones, ginkgolides, and bilobalides whose structures were established by Nakanishi and his coworkers (Nakanishi 2005, Strømgaard and Nakanishi 2004). Ginkgolides possess improvement of memory, increased blood circulation, and beneficial effects to suffer of Alzheimer's disease. This class of the molecules has been studied for its potential to act as a platelet-activating factor receptor antagonist. Ginkgolide B (**12**) has been studied for its potential to reduce migraine frequency and it functions as a selective antagonist of glycine receptors based on noncompetitive inhibition for neurological system that this compound forms and is used in treatment for cerebrovascular disorders. Ginkgolides A-C (**11**–**13**) are platelet activation factor antagonists which are considered to contribute to their effects on cognitive disorders and these trilactones inhibit NF-kB signaling and iNOS activation. Ginkgolide A (**11**) could be explored for a potential role in managing vascular complications in diabetes, since it improved high glucose-induced vascular inflammation via modulation of the STAT-3-pathway.

17.2.18 *Helianthus* (Asteraceae)

Four species belonging to this genus are listed in Table 1. The representative species is the sunflower (*H. annuus*). The seeds of the *Helianthus* have been used as foods and food additives. This genus is a rich source of kaurene diterpenoids possessing anti-inflammatory activity.

17.2.19 *Helichrysum* (Asteraceae)

The genus *Helichrysum* consists of an estimated 600 species of flowering plants in the family Asteraceae. The type species is *Helichrysum orientale*. This genus is also a rich source of kaurane diterpenoids.

17.2.20 *Hypoestes* (Acantaceae)

The *Hypoestes* is a flowering plant genus with 150 species which are widely distributed in the tropical and subtropical region around the Indian Ocean. Many species that belong to this genus have various bio- and pharmacological activities, such as antimicrobial, antifungal, antioxidant, antitrypanosomal, and antileishmanial, and used as folk medicines, for treating many diseases, breast, liver, heart, and respiratory infection, anemia, scabies, skin disorders, typhoid, hypertension, and gonorrhea. The *Hypoestes* are rich sources of diterpenoids, labdanes, furanolabdanes, isopimaranes, dolabellanes, and especially fusicoccanes, such as hypoestenonols from *H. forskalei* which exhibited antifungal and antimalarial activity (Al Haidari 2018).

17.2.21 *Isodon* (Lamiaceae)

The *Isodon* is a group of flowering plants in the family Lamiaceae described as a genus in 1840. It is native to tropical and subtropical parts of the Old World, primarily Asia but two species are from Africa. Many of the species are endemic to China. Of 150 species of *Isodon*, nearly 70% is distributed in tropical and subtropical Asia. The *Isodon* is a genus of mint relatives whose member species has also been called *Rabdosia* or *Plectranthus*. Primarily Asian in origin, *Isodon* consists of about 100 species of *Salvia*-like plants a few of which have potential in the woodland garden. Ndoile (2020) reported 131 of diterpenoids from *Isodon* species. Sun et al. (2006) and Liu et al. (2017) described the review articles concerning diterpenoids from the *Isodon* species and their biological activity with 469 *ent*-kaurene as well as ent-gibberellanes, abietanes, and *ent*-abietanes, and the other bicyclic and tricyclic diterpenoids and the *ent*-kaurane structures categorized as 1) C-20 non oxygenated *ent*-kauranes, 2) C-20 oxygenated kauranes, (mono-epoxy-kauranes, diepoxy-*ent*-kauranes, C-20 oxygenated non-epoxy ring ent-kauranes), 3) 6,7-seco-*ent*-kauranes (enmein type, spiro-lactone type), 4) 8,9-seco-*ent*-kauranes, 5) 8,15-seco-*ent*-kauranes and 15,16-seco-*ent*-kauranes, 6) 7,20-cyclo-*ent*-kauranes, 7) *ent*-kaurane dimers, and 8) miscellaneous *ent*-kauranes, and 290 references. The isolated *ent*-kauranes and their related compounds possess antimicrobial, cytotoxic, antitumor, and antiviral activity.

17.2.22 *Nepeta* (Lamiaceae)

The *Nepeta* is native to Asia, Africa, and Europe, and North America and Mediterranean regions with over 250 species known. Several *Nepeta* species have

traditionally been used as diuretic, diaphoretic, antitussive, antibacterial, antifungal, antispasmodic, antiasthmatic, febrifuge, sedative, spice and herbal tea, as well as food and food supplements, an ingredient of vegetable soup, traditional foods, and used for colds, flu, and bronchitis. Nepetalactones are characteristic monoterpenes for some *Napeta* species as discussed in the dietary monoterpenoids (▶ Chap. 16, "Dietary Monoterpenoids"); however, a number of this species produced different diterpenoids, mainly abietanes and pimaranes (Yilmaz et al. 2016).

17.2.23 *Phlomis* (Lamiaceae)

The *Phlomis* is a genus of over 100 species of herbaceous plants, subshrubs, and shrubs in the family Lamiaceae, native from the Mediterranean region east across central Asia to China. Common names include Jerusalem sage and lamp wick plant. The *Phlomis* mainly produces abietanes and labdanes, and several species have been used as herbal tea.

17.2.24 *Pinus* (Pinaceae)

The Pines are any conifers in the genus *Pinus* of the family Pinaceae. The *Pinus* is the sole genus in the subfamily Pinoideae. About 126 species belonging to this genus have been known with 35 unresolved species. Pine is one of the more extensively used types of wood used as lumber. Almost all of the pine trees elaborate not only mono- and sesquiterpenoids but also diterpenoids of which abietanes and kaurenes are predominant constituents. The hexane extracts of the leaves of some of pine trees contains 80% of *ent*-kaurene in total yield. Some pine nuts are edible and leaves used as herbal tea.

17.2.25 *Plectranthus* (Lamiaceae)

The *Plectranthus* is a group of flowering plants in the family Lamiaceae. This genus grown in Southern and tropical Africa and Madagascar has 300 species of which 62 species are reported to be used as medicines, ornamentals, foods, flavors, and fodder (Lukhoba et al. 2006). Many species are rich sources of abietanes and their related compounds indicating antioxidant, antimalarial, antifungal, and cytotoxic effects.

17.2.26 *Podocarpus* (Podocarpaceae)

The *Podocarpus* is a genus of ever green or shrubs conifers, the most numerous and widely distributed of the podocarp family, Podocarpaceae, brightly colored receptacle at maturity. There are about 100 species in the genus depending on the

circumscription of the species. The *Podocarpus* species produce nagilactone diterpenoids, such as nagilactone A (**14**) which are naturally very rare constituents, restricted in this genus, together with abietanes. In spite of the presence of such nagilactone compounds and the other diterpenes found in this genus, little has been known of their bio- and pharmacological activity except for the constituents of *P. nagi* and the Japanese yew, *P. macrophyllus*.

17.2.27 *Premna* (Lamiaceae)

The *Premna* is a genus of flowering plants in the mint family, Lamiaceae, first described for modern science in 1771. It is widespread through tropical and subtropical regions in Africa, southern Asia, northern Australia, and various islands in the Pacific and Indian Oceans. The main diterpenoids of this genus are highly oxidized abietanes, clerodanes, and labdanes possessing antimicrobial activity.

17.2.28 Propolis (Bee Glue)

Propolis is one of rich sources of pharmacologically active diterpenoids in nature. It is a resinous material that is mostly collected by honey bees (*Apis mellifera* L.). Propolis has been used as many functional foods and cosmetics. Propolis collected in the different locations possess different diterpenoids, especially abietanes and labdanes, which show antimicrobial, antiviral, antifungal, antiparasitic, anti-inflammatory, cytotoxic effect, and immunomodulatory effect (Aminimoghadamfarouj and Nematollahi 2017).

17.2.29 *Rabdosia* (Lamiaceae)

The *Rabdosia* includes over 100 species mainly found in Japan, China, eastern Africa, Indian, Himalaya, and southern Asia. Many species of this genus have been used as the traditional medicine for the treatment of several diseases, such as respiratory and gastrointestinal bacterial infection. *R. japonica,* which has been used for treatment of hepatitis, gastricism, mastitis, and coughing, was highly studied phytochemically by Japanese and Chinese researchers and shown to have antitumor, antioxidant, anticoagulant, antimicrobial, and anticomplementary activities . It contains a bitter principle, enmein (**15**) and many other related kaurene type diterpenoids (Xiang et al. 2015).

17.2.30 *Rosmarinus* (Lamiaceae)

Salvia rosmarinus, commonly known as rosemary, is a herb with woody perennial fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers, native to the Mediterranean region. Until 2017, it was known by the scientific

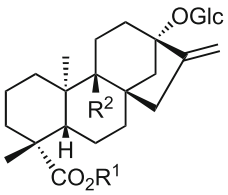
name *Rosmarinus officinalis*, now a synonym. It is a member of the mint family Lamiaceae, which includes many other herbs. The aerial parts have been used as spice and herbal tea as well as cosmetics and flavoring foods. It contains carnosol (**16**), rosmanol (**17**), and isorosmanol (**18**) and their related compounds which have various kinds of bio- and pharmacological activity such as anticancer, antifungal, and antidiabetic.

17.2.31 *Rubus* (Rosaceae)

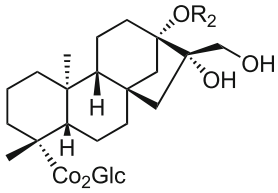
In Guan Xi province in Main China, the Chinese tea plant, *Rubus suavissimum* belonging to the Rosaceae is grown. Its leaves possess strong sweet taste and used as beverage (sweet tea, tian-cha). Its sweet taste is responsible for the diterpene glycoside, named rubsoside (**19**) whose aglycone is the same as that of stevioside (**20**); however, one glucose is absent. Enzymatic transformation of stevioside gives rubusoside which is 130 times sweeter than sucrose. Rubusoside is an excellent solubilizing agent. It can enhance the solubility of important pharmaceutics, such as curcumin, liquiritin, teniposide, and etoposide. In addition to rubusoside, *R. suavissimum* elaborates rubusoside-related glucosides (**19a–i**) (Chart 4) (Kinghorn et al. 2010).

17.2.32 *Salvia* (Lamiaceae)

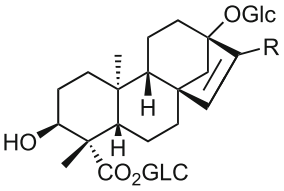
The *Salvia* is the largest genus in the family of the Lamiaceae plants and they are distributed mainly in South and Central America (500 species), central Asia and Mediterranean region (250), and eastern Asia (90). Wu et al. (2012) reported that 730 constituents were isolated from 134 *Salvia* species of the over 1000 species. *Salvia* species produce an incredible numbers of diterpenoids, abietanes (365), possessing simple structures and its derivatives are highly oxygenated, together with clerodanes (138), pimaranes (20), and labdanes (18) and among others. *Salvia* also elaborates with sesqui-, C-23-, C-25-terpenoids and triterpenoids and flavonoids. *Salvia officinalis* grown in the Mediterranean regions has fully been studied chemically and a number of diterpenoids especially abietanes were isolated and their structures as well as bio- and pharmacological activity such as anticancer, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, antimutagenic, antidementia, hypoglycemic, and hypolipidemic effects were reported (Ghorbani and Esmmaeilizadeh 2017). In China, a number of *Salvia* species have been used in Traditional Chinese Medicine (TCM). *Salvia miltiorrhiza* known as red sage (丹參; pinyin: *dānshēn*) has been used for the treatment of cardiovascular and cerebrovascular disorders. It has also been used for circulation problems, chronic liver disease, sleeping trouble, and wound healing as well as skin problems, for example, acne, psoriasis, and eczema. This herb produces many abietanes, such as tanshinones (**21**) many of which possess *ortho* or *para*-quinone moieties in the molecule, and show significant pharmacological activity such as anticancer, antioxidant, antibacterial, neurodegenerative, anti-inflammatory, kidney and liver



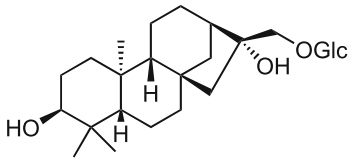
rubusoside (**19**): R^1=Glc, R^2=H
steviol monoside (**19b**): R^1=R^2=H
suavioside B (**19c**): R^1=Glc, R^2=OH



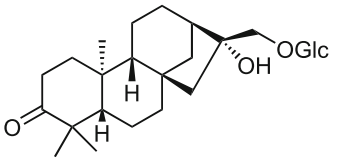
suavioside G (**19d**): R^1=H, R^2=Glc
suavioside J (**19e**): R^1=OH, R^2=H



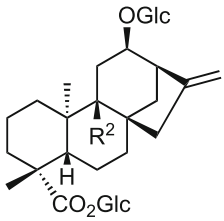
suavioside H (**19f**): R= CHO
suavioside I (**19g**: R= CH_2OH



suavioside A (**19a**):



ent-3-oxo-16β-hydroxy-kaurane-17-*O*-β-glucoside (**19h**)



cussoracoside (**19i**)

Glc= β-D-glucopyranosyl

Chart 4 Sweet diterpene glucoside, rubusoside (**19**) and its related compounds (**19a**–**h**) from the leaves of *Rubus suavissimus*, and cussoracoside (**19i**) from *Cussonia racemosa* (Kinghorn et al. 2010)

protective, anti-HIV, and antidiabetic activity (Jiang et al. 2019). *S. hispanica* (Chia) which began to be used in human food ca 3500 BC is distributed in Mexico to Guatemala. It is now cultivated in America, Australia, and Southeast Asia to obtain seeds which have been used as "Chia" as foods and seed oil. *S. hispanica* contains neoclerodane diterpenoids named hispanins A-J and salvihispin A-G and their related compounds. Hispanins D, F, I, and 12-hydroxyhautriwaic lactone and bacchotricuneatin A as well as salvihispin F show cardioprotective effects (Fan et al. 2019, 2020).

Sclareol (**22**) which was isolated from *Salvia sclarea* has been used major as a raw material for fragrance industry to produce cosmetics and food flavor (Fahlbusch

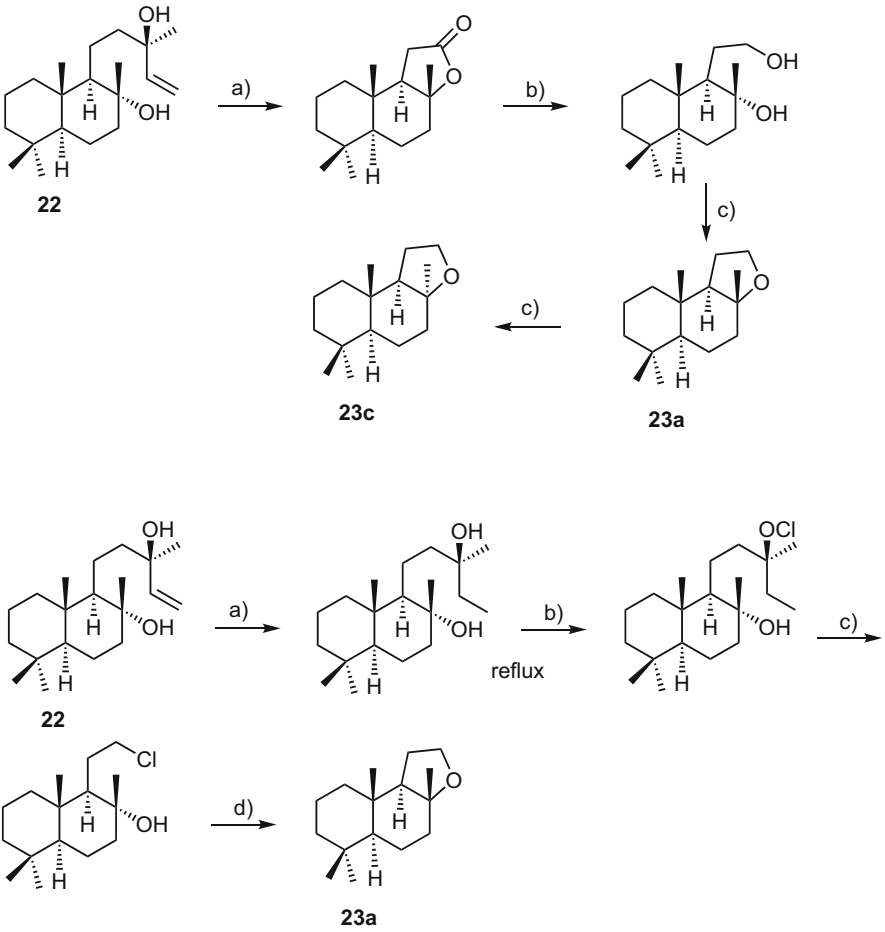


Fig. 1 Two hemisyntheses of ambrox (**23a**) from sclareol (**22**) (Decorzant et al. 1987). (**a**) CrO_3/AcOH, (**b**) LiAlH4/Ether, (**c**) 3-naphthalenesulfonic acid. (**a**) 5% Pd-C/EtOH, (**b**) aq.NaOCl/CCl_4, (**c**) 30–35 °C/3 h/reflux, (**d**) NaH/THF/3 h

et al. 2007). Sclareol is an important starting material to make ambrox (=ambroxide) (**23a**) originated from ambrain (Decorzant et al. 1987) (Fig. 1). The liverwort, *Porella perrottetii* produces a large amount of a labdane diterpenoids (**23b**) from which ambrox (**23a**) was synthesized in very good yield (Fig. 2) (Asakawa et al. 2013).

17.2.33 *Sideritis* (Lamiaceae)

The genus *Sideritis* contains more than 320 perennial and annual vegetable species widely distributed in the Mediterranean, the Balkans, the Iberian Peninsula and

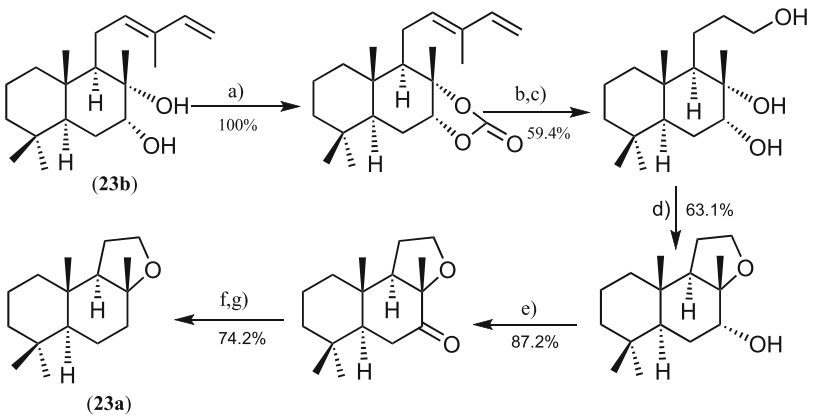


Fig. 2 Hemisynthesis of ambrox (**23a**) from labda-12,14-diene-7α,8α-diol (**23b**) isolated from liverwort, *Porella perrotteniana* (Asakawa et al. 2013). (**a**) $CH_3COCOOCCl_3/Py/CH_2Cl_2$, (**b**) O_3/CH_2Cl_2, (**c**) $LiAlH_4$, (**d**) $H^+/CH_3NO_2/p$-TsOH, (**e**) CrO_3-H_2SO_4, (**f**) $TsNHNH_2$, (**g**) $NaBH_3CN$

Macaronesia, Central Europe, and temperate Asia region. They are traditionally used "tea" for feeding, flavoring agents, and folk medicine as anti-inflammatory, anti-ulcerative, antimicrobial, antioxidant, antispasmodic, anticonvulsant, analgesic, and carminative agents (Burgos et al. 2011). The *Sideritis* produces *ent*-kauranes as predominant diterpenes.

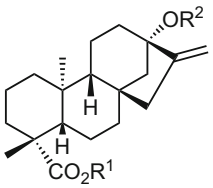
17.2.34 *Stachys* (Lamiaceae)

The *Stachys* is one of the largest genera in the family Lamiaceae and there are about 350–450 species. The type species for the genus is *S. sylvatica.* The distribution of the genus covers Asia, Africa, Australasia, and North America. *S. officinalis* was the most important medicinal herb in England. *S. affinis* is grown for its edible tuber. Several species are cultivated as ornamentals. The abietanes, kauranes, and labdanes have been found as the major constituents from the *Stachys*.

17.2.35 *Stevia* (Asteraceae)

In recent decades, the request for no caloric natural sweeteners in the food industry has increased the extraction of polysaccharides and other sweeteners isolated from plants, fungi, and algae due to their various biological activities. One of the most important no caloric plant sweeteners are several ent-kaurene glycosides with steviol (**24**) as a common aglycone isolated from *Stevia rebaudiana* belonging to the Asteraceae which is native to subtropical and tropical South and Central America. Among glycosides, stevioside (**20**) is the most abundant followed by rebaudioside A (**25**). The former diterpene glycoside is 140 times sweeter than sucrose, while the

Chart 5 Stevioside (**20**) and its related compounds (**20a**, **24**, **25**–**25f**) from the leaves of *Setevia rebaudiana* (Kinghorn et al. 2010). Glc = β-D-glucopyranosyl, Rha = α-L-Rhamnopyranosyl, Xyl = β-D-xylopyranosyl



stevioside (**20**): R^1=Glc, R^2=Glc-Glc (2-1)

steviolbioside (**20a**): R^1=H, R^2=Glc-Glc (2-1)

steviol (**24**): R^1=R^2=H

rebaudioside A (**25**): R^1=Glc, R^2=Glc-Glc (2-1), Glc (3-1)

rebaudioside B (**25a**): R^1=H, R^2=Glc-Glc (2-1), Glc (3-1)

rebaudioside C (**25b**): R^1=GLc, R^2=Glc-Rha (2-1), Glc (3-1) (=dulcoside B)

rebaudioside D (**25c**): R^1=Gluc-Gluc (2-1), R^2=Glc-Glc (2-1), Glc (3-1)

rebaudioside E (**25d**): R^1=GLc-Glc (2-1) R^2=Glc-Glc (2-1)

rebaudioside F (**25e**): R^1=GLc-Glc (2-1) R^2=Glc-Xyl (2-1), Glc (3-1)

dulcoside A (**25f**): R^1=GLc, R^2=Glc-Rha (2-1)

latter compound is 240 times sweeter than sucrose. Rebaudioside A possesses a better quality of sweetness. Now it is widely and legally consumed by millions of people from China, Korea, Israel, and especially Japan where the herb and the extract have been used since 1970s in numerous food products with an extensive safety taste, including soy sauce, pickles, boiled fish paste, and soft drink without any health risk. In 2006, Japan consumed more *Stevia* than any other country with stevia accounting for about 40% of the sweetener market. Steviosides are stable to remain sweet taste in processed foods and in hot soft drink like coffee (Goyal et al. 2010). *S. rebaudiana* produces not only stevioside (**20**), but also many similar *ent*-kaurene glucosides (**20a, 24, 25a–f**) (Chart 5) (Kinghorn et al. 2010).

17.2.36 *Solidago* (Asteraceae)

The aerial parts of *Solidago* species contain not only mono- and sesquiterpenoids while their roots are a rich source of clerodane diterpenoids. However, these

species are only used as the medicinal herbs and their bioactivity remained to be clarified.

17.2.37 Taxus (Taxaceae)

The *Taxus* is known as "Yews" a genus of evergreen coniferous trees belonging to the family Taxaceae mainly distributed in the northern hemisphere. All species of yew contain highly poisonous taxine alkaloids, although ripe fruits are sweet and edible. *Taxus brevifolia* (Pacific yew) and *Taxus canadensis* (Canadian yew) were the initial sources of paclitaxel (taxol) (**1**), possessing taxane diterpene skeleton which is one of the most important chemotherapeutic drug used in breast and lung cancer treatment. Since the discovery of paclitaxel and its related compounds, the chemical studies on secondary metabolites of different *Taxus* trees have resulted in the isolation of a great number of new taxoids possessing the interesting pharmacological activity (Wani et al. 1971; Suffness 1995; Kingston et al. 1993; Appendino 1995). Other yew species, for example, *Taxus mairei, T. wallichiana*, and *T. yunnanensis*, contain many similar taxane compounds with anticancer activity. Docetaxel, an analogue of paclitaxel, originated from *Taxus baccata* (European yew). The Japanese yew has been used to treat diabetes and promote diuresis and as an emmenagogue. From *T. cuspidata* about 120 taxoids have been isolated. Among the isolated toxoids, several non-taxol type compounds have been shown to reduce calcium chloride induced depolymerization of microtubules, increase cellular accumulation of vincristine in multidrug-resistant tumor cells, and showed strong cytotoxicity (Shigemori and Kobayashi 2004). Wang et al. (2011) reported a review article entitled "Natural taxanes: developments since 1823" which cited 513 references with more than 500 taxoid compounds and their bio- and pharmacological activity.

17.2.38 *Taxodium* (Cupressaceae)

The *Taxodium* species belongs to the Cupressaceae family and they are closely related morphologically to the Japanese *Cryptomeria japonica* and Chinese swamp cypress, *Glyptostobus pensilis*. Only a few *Taxodon* species have been known, *T. mucronatum, T. ascendens*, and *T. districhum* which produce cytotoxic abietane diterpenoids, taxodion, taxodone, taxoquinone (=7β-hydroxyroyleanone), sugiol, Δ^3-dehydrosugiol as well as labda-8(20),13-dien-15-oic acid and metasequoic acid which indicate antifilarial activity.

17.2.39 *Teucrium* (Lamiaceae)

About 300 species of the *Teucrium* belonging to the Lamiaceae are distributed in the northern temperate and subtropical regions, mainly in the Mediterranean region.

Many species of this genus have been used for over 2000 years in traditional folk medicines as diuretic, diaphoretic, hypoglycemic, hypolipidemic, antioxidant, antipyretic, anti-inflammatory, antiulcer, antitumor, and antibacterial crude drugs. Several species have been used as tonic and to treat stomachic, intestinal disorders, and colds, and for feminine sterility. One of the most common and highly studied species in this genus is *T. chamaedrys* named germander which is native to Europe and used in the treatment of digestive and respiratory diseases, abscesses, gout as well as astringent infusion on the gums and for wound healing.

Hydro-alcoholic extract has been used as approved substances in the preparation of flavored wines, bitters, and liqueurs. More than 220 diterpenoids all of which clerodane or neoclerodane skeleton have been isolated from the *Teucrium*. Many of them possess clerodane 20,12β-lactone moiety. The extracts of some species were exhibited to potentiate the cytotoxic and proapoptotic effects of anticancer drugs vincristine, vinblastine, and doxorubicin, against a panel of carcinogenic cell lines (Rajabalian 2008).

17.2.40 *Alpinia, Amonum, Elettaria, Curcuma*, and *Zingiber* (Zingiberaceae)

The *Alpinia, Amonum, Elettaria, Curcuma*, and *Zinger* belong to the Zingiberaceae family. The *Alpinia* is the largest genus in this family, with about 230 species many of which have been used as foods and spices as well as flower decoration. In Table 1, several *Alpinia* species having bio- and pharmacologically active diterpenoids are listed. *A. galanga* exhibited potent antimicrobial activity which is responsible for 8(17),12-labdane-15,16-dilal (**29**) (Widyowati and Agil 2018).

The seeds of cardamon or cardamom originated from the *Amonum* or *Elettaria* species have been used as foods, food additives, spices, and herbal tea. *E. cardamomum* (小豆蔻) and *A. villosum* var. *xanthioides* (縮砂) have been used as Kampo medicines. 8(17),12-Labdadien-15,16-dial (**29**) obtained from *E. cardamomum* and *Curcuma mangga* indicates potent antimicrobial activity. Calcaratarin A, communic acid (**3**), and copallic acid (**30**) also inhibit COX-II expression. In Korea, *A. villosum xanthioides* and *A. tsaoko* have been used as traditional tea.

Many *Curcuma* species possess food and nutritional value and are chemically studies extensively. The rhizomes of *Curcuma angustifolia, C. caulina, C. leucorrhiza*, and *C. xanthorrhiza* are good sources of food supplements. The rhizomes of *C. aeruginosa, C. amada, C, aromatica C. longa, C. pirreana, C. pseudomontana, C. purpurascens, C. xanthorrhiza*, and *C. zedoaria* are also used as dye, spice, and food flavoring, and coloring agent in food preparation. In fluorescence, tuberous roots, and rootstocks of *C. angustifolia, C. amada, C. australis, caulina,* C. *manga*, *C. pierreana*, and *C. pseudomontana* have been reported to be used as food appetizers, vegetables, and culinary preparations or making pickles, flavoring against steamed and baked fishes (Rajkumari and Sanatombi 2017). The crude extract of *C. manga* shows cytotoxic effects against MCF-7, KB, A549, Ca Ski, and HT-29 cell lines. The isolated diterpenoids, (*E*)-labda-8(17),12-diene-15,16-dial (**29**) and zerumin A (**69**) possessed

high cytotoxic effects against all six cancer cell lines (Malek et al. 2011). *C. heyneana* also produces the same labdane dial (**29**) which shows antimicrobial activity.

Zingiber officinale is the most widely used species as foods, vegetables, food additives, spices, and beverage among the Zingiberaceae family. This plant has been used as medicinal plant to cure many diseases. The first biologically active compound, galanolactone (**26**), a labdane epoxy-γ-lactone, possessing antagonist at 5-HT3 receptor was isolated from *Z. officinale* (Huang et al. 1991). The same lactone was found in some *Alpinia* species *A. galanga* and *A. katsumandai* which have been used as foods and beverage including antimicrobial, antifungal, and other pharmacological activity (see Table 1).

The rhizome of another *Zinger* species, *Z. mioga*, which is smaller than that of *Z. officinale* is also used as foods, vegetables, and food additives. The whole plant exhibited anticancer, antihypertensive, anti-inflammatory, and antiviral activity and used to treat abnormal and irregular digestion, fever, and hematemesis and so on. The flower buds of *Z. mioga* have been used as spice or pickles in Japan because of its characteristic pungent and pleasant odor. Abe et al. (2004) reported the isolation of three labdane dialdehydes, miogadial (**27**), which is responsible for the pungent flavor, galanal A (**28**) and B from the flower buds of *Z. mioga*. These three diterpene dialdehydes showed antimicrobial activity against Gram positive bacteria.

17.2.41 *Ziziphora* (Lamiaceae)

The *Ziziphora* is a genus of annual or perennial herbs or subshrubs in the family Lamiaceae and there are about 20 species distributed in Southern and Eastern Europe, North-West Africa, and Asia to the Himalayas and Altai mountains. *Ziziphora* has aromatic leaves which contain antimicrobial compounds and used as herbal tea.

17.3 Distribution of Diterpenoids and Related Compounds in Spore-Forming Plants and Mushrooms

17.3.1 Algae

Many types of sea algae (=sea weeds) are used as food, food additives, processed foods, and even beverages (algal tea with crude extract of *Plunus mume*) especially Japan, China, and Korea. They are also rich source of biological and pharmacological constituents with therapeutic properties. They have been established as healthy foods and food additives that are rich in dietary fibers and minerals. Many of them have been used as traditional folk medicines and drugs.

The common edible green, brown, and red algae are as follows:

Green algae: *Caulerpa lentillifera* (sea grape or green caviar), *Chlorella, Monostroma nitidum* (Hitoegusa* Japanese name), *Ulva compressa* (Aonori*), *U. intestinalis*, and *Ulva* species (sea lettuce, Aosa*)

Brown algae: *Alaria esculenta, Cladosiphon okamuranus* (Mozuku*), *Ecklonia cava, Eisenia bicyclis* (Arame*), *Fucus spiralis, F. vesiculosus, Himanthalia elongate, Laminaria digitata, Mastocarpus papillatus, Nereocystis luetkeana, Pelvetia canaliculata, Postelsia palmaeformis, Saccharina japonica* (Konbu*), *S. latissima, S. cinetum, Sargassum echinocarpum, S. fusiforme* (Hijiki*), *S. myriocysum, S. swartzii, S. vulgare, Undaria pinnatifida* (Wakame*), and *U. undarioides*

Red algae: *Chondrus crispus, Eucheuma spinosum, E. cottonii, Gelidiella acerosa, Gelidium crinale* (Tengusa*), *Gracilaria edulis, G. corticata, Mastcarpus papillatus, Mastocarpus stellatus, Neopyropia tenera* (Asakusanori*), *Palmaria palmata, Porphyra laciniata, P. umbilicalis*

El Gemal (2010) reported the review entitled "biological importance of marine algae" with 272 references and 386 structures among which 46 of diterpenoids and their biological activities were described. The similar review article concerning algal benefits; nutrition, medicine, pharmaceutics and cosmetics, and historical story of algae as foods, medicines, food industries and nutrition (polysaccharides, fifer, proteins and amino acids, lipids and fatty acids, minerals, vitamins and cosmetics), as well as classification of algae and pharmacological activity of each isolated secondary product including diterpenoids with mostly antibacterial, antiviral, and cytotoxicity of 102 species has been reported using 37 references (Anis et al. 2017).

17.3.1.1 *Ulva* (Green Algae)

Ulva fasciata, a green algae, contains seven labdane diterpenoids among which two labda-14-ene-3α,8α-diol and labda-14-ene-8α-hydroxy-3-one showed antibacterial activity against *V. alginolyticus* and *Vibrio parahaemolyticus* (Chakraborty et al. 2010). *Caulerpa lentillifera* is tasty green algae which grows in Okinawan cost. It contains unidentified terpenoids and steroids which showed antioxidant and antimicrobial activity (Asakawa unpublished results).

17.3.1.2 *Dictyota* (Brown Algae)

It is a genus of brown seaweed in the family Dictyotaceae. The species are predominantly found in tropical and subtropical seas, and are known to contain numerous chemicals (diterpenes) which have potential medicinal value. As at the end of 2017, some 237 different diterpenes had been identified from across the genus. Among the brown algae, *Dictyota* belonging to the Dictyotaceae are rich sources of diterpenoids and some of them are edible, such as *D. dichotoma*. Chen et al. (2018) summarized the distribution of diterpenoids with 90 references up to the end of 2017. A total of 233 diterpenoids were described, and they are divided into three groups: prenylguaianes, germacranes, cadinanes and epielemanes (Group 1), dolabellanes, such as

3*E*,7*E*,18-dolabellatrien-14-one (**31**) *from D. dichotoma,* dolastanes, 1(15),8-dolastadiene-2β,14β-diol (**32**) from *D. linearis,* secodolastanes, dictyoxetanes (Group 2), xenicanes, 4,18-dihydroxy-1(9),6,13-xenicatrien-19-al (**33**) from *D. dichotoma*, crenulidane, dichotomane, and crenulane (Group 3), among which the group 2 (120) is composed of almost 50% of the total diterpenoids (233) of the *Dictyota* species, and most of the isolated diterpenoids that originated from *D. dichotoma* possess antibacterial, antifungal, antiviral, cytotoxic, anti-inflammatory activity.

17.3.1.3 *Dilophus* (Brown Algae)

The *Dilophus* is distributed in the tropical zone of both hemispheres. Most of diterpenoids found in this genus share the same structural skeleton with those of *Dictyota* because the *Dilophus* is taxonomically similar to *Dictyota* mentioned above. The most characteristic chemical feature of this genus is the presence of cubebane-skeletal diterpenoids which show potent antibacterial and antifeedant activities. Xia et al. (2020) reported the review article of this genus including 124 diterpenoids and 63 references.

17.3.1.4 Red Algae

One of the most consumable red algae in the world is *Gelidium crinale* from which agar-agar is produced and it has been used as many foods, food additives, and processed foods; however, it does not contain any diterpenoids. The *Laurencia* species are rich sources of mono-, sesqui-, and diterpenoids, many of which possess chlorine and bromine atoms; however, these algae have not been used as foods.

17.3.2 Lichens

There are about 20,000 species of lichens in the world; however, several species, such as *Bryoria fremontii* (wila), *Cetraria islandica* (Iceland moss), *Cladonia rangiferina*, *Parmelia perata* (Kalpasi or black stone flower), *Umbilicaria esculenta*, are edible foods and some of them have been used as beverage (herbal tea). *C. islandica* was an important source of food for northern European, and was cooked as a salad, soup bread, or pudding. *B. fremontii* was an important food in North America. *Umbilicaria esculenta* is rare lichen which is used as traditional food in Japan and Korea. However, these edible lichens lack diterpenoids, and many aromatic compounds have been found as major components and their antioxidant property and cytotoxicity are reported. Only one edible lichen *C. rangiferina* produced several diterpenoids, abietanes and labdanes, and a pimarane derivative of which only abietane derivatives, hanagokenols A and obtuanhydride, sugiol and monbretol showed antimicrobial activity (Yoshikawa et al. 2008). Huneck and Yoshimura (1996) reported that rimuene containing lichen, *Evernia prunastri* (oak moss), shows antiseptic, emollient, and expectorant activity. Decocted *Letharia vulpina* (wolf lichen) which contains manool (**34**) and sandaracopimaric acid (**35**) was used as beverage to stop bleeding, stomach disorder, and poultice for swelling. *Ramalina hierrensis* and *R. tumidula* contain (-)-*ent*-kauran-16α-ol (**36**) and (-)-sandaracopimaric acid, respectively.

17.3.3 Bryophytes

Bryophytes are divided into three classes, Marchantiophyta (6000), Bryophyta (14,000), and Anthoceratophyta (300 species), among which Marchantiophyta (liverworts) contains cellular oil bodies. About 24 species of bryophytes have been used as medicinal plants especially in China. Among bryophytes, clerodanes, kaurenes, labdanes, cembranes, cyathanes, dolabellanes, fusicoccanes, pimaranes, phytanes, sacculatanes, sphenolobanes, trachylobanes, verticillanes, vibsanes, and viscidanes diterpenoids have been found in liverworts as major components and their absolute configuration and biological activity are reported; however, nobody knows whether these diterpene containing liverworts are edible or not. Only one large thalloid liverwort, *Marchantia polymorpha* which shows various biological activity: antimicrobial, antifungal, antioxidative, diuretic, potent antitrypanosomal, muscle relaxant, and tubulin polymerization and nitric oxide production inhibitory activity, is edible liverwort, and it is used as herbal tea and liqueur. It contains only labda-3,13(*E*)-dien-15-ol as a minor component (Asakawa 1982, 1995, Asakawa et al. 2013).

17.3.4 Ferns

Ferns comprise more than 12,000 species spread among 250 different genera. Baskaran et al. (2018) described in their review article entitled "A review of the use of pteridophytes for treating human ailments" 66 species including bio- and pharmacological activity citing 300 references. The young shoots of several ferns, *Athyrium esculentum*, *A. filix-femina*, *Matteuccia struthiopteris, Osmunda cinnamomea*, *O. japonica*, *O. regalis*, *Polystichum munitum*, *Pteridium aquilinum*, and *Stenochlaena palustris* are edible as a cooked leaf vegetable. Among which *O. japonica* biosynthesizes three *ent*-kauranes, such as *ent*-kauran-16(*S*),17-hydroxy-19-oic acid (**37**) possessing cytotoxic effects against HeLa and HepG2 cancer cell lines (Bowen et al. 2020) and *P. munitum* shows anti-inflammatory and anticancer activity for dandruff (Cao et al. 2017).

Flavonoids, hydroxycinnamic acid, and anthocyanin antioxidants have been found in some edible and many inedible ferns. The sprophyte of *Equisetum arvense* and roots of *P. aquilium* have been used as cooked vegetable and production of highly qualified starch, respectively. However, these species do not contain diterpenoids. On the other hand, the bitter fern, *Gleichenia quadripartita* contains 15 new labdane diterpene glycosides (Socolsky et al. 2007).

Dicranopteris dichotoma (=*Dicranopteris linearis)* has been used for parasites, skin ulcer, cut, and cold. It produces three tetranorclerodanes, such as aylthonic acid, and five *ent*-clerodanes such as 6(*S*),13(*S*)-cleroda-3,14-dien-6,13-diol which shows HIV inhibitory activity (Li et al. 2007).

The fern species, *Pteris* belonging to the Pteridaceae, are rich sources of kaurenes and kauranes and their glycosides. In addition, a few labdanes have been also confirmed as a small amount (Cao et al. 2017). Especially *P. cretica, P. dipar, P. plumbaea*,

and *P. semipinnata* contain these diterpenoids possessing various biological activities. For example, *P. dipar* contains 5β,11β,12β-trihydroxy-15-oxo-*ent*-kaur-16-en-19-oic acid (**38**) indicating potent cytotoxicity against KB cell. *ent*-11α-Hydroxy-15-oxokaur-16-ene (**5**) which was isolated from the Chinese medicinal fern *P. semipinnata* used for the treatment of venomous snake bites shows significant cytotoxicity and anticancer activity (Cao et al. 2017). It also indicates apoptosis in human colon cancer HT-29 and MNK-45 cells as well as cytotoxicity on the gastric cancer. The same kaurenoic acid also inhibits the proliferation of the human lung cancer cell lines (A549, NCH23, and CRL-2066), hepatocellular carcinoma (HCC), and lung adenocarcinoma cells. 7,9-Dihydroxy-15-oxo-*ent*-kaure-16-en-19,6-olide and 7,11-dihydroxy-15-oxo-*ent*-kaur-16-en-19,6-olide also inhibit lung adenocarcinoma cells. *P. multifida* which show antidysenteric, anti-inflammatory, antioxidant, antipyretic, antirheumatism, and detoxicant activity has been used for acne, appendicitis, and bleeding as the Chinese medicine contains 2β,16α-dihydroxy-*ent*-kaurane, 2β,15α,18-trihydroxy-*ent*-kaur-16-ene, and 2β,6β,16α,17-tetrahydroxy-*ent*-kaurane (Ge et al. 2008).

17.3.5 Mushrooms

More than 15,000 mushrooms are distributed in the world. Among them, 10 to 14% of the total mushrooms are edible as foods, vegetable, beverages, and processed foods in East Asian counties, China, Japan, and Korea. The others are nontoxic but inedible (too hard to chew) and toxic. Some nontoxic and inedible mushroom contains a big amount of spider-female pheromone and antimicrobial and antiviral compounds (Quang et al. 2006). About 700 species of the mushrooms have been used as traditional medicines and crude drugs. The distribution of diterpenoids is rare in mushrooms and fungi; however, a number of cyathane diterpenoids have especially been found in edible and inedible mushrooms belonging to the *Cyathus* and *Sarcodon* species. *Hericium* species are mushroom belonging to the family Hericiaceae and have been known as Chinese medicine or food without harmful. In particular, *Hericium erinaceum* (Yamabushitake, in Japanese) which is marketed as supplement, contains erinacine A-C (**39–41**), P (**42a**), and Q (**42b**) which exhibited nerve-growth-factor synthesis stimulatory and κ-opioid receptor agonist activities (Kawagishi et al. 1994, 1996a, b, 2006; Kenmoku et al. 2000, 2001, 2002, 2004).

The review concerning structural elucidation and bioactivity of erinacines from the fruiting body and mycelium of *Hericium erinaceum* have been reported by Ma et al. (2010). Xia et al. (2012) reported the structures of 109 cyatanes and its related compounds from *Cyathus* species with 96 references and their biological activities such as antimicrobial, anti-Methicillin-resistant bacterial, *Staphylococcus aureus*, osteroclast-forming suppressing agonistic toward the κ-opioid receptor, anti-inflammatory, and cytotoxic effect as well as stricture activity relationship in detail. The similar review concerning secondary metabolites of mushrooms including cyathane diterpenoids with 43 structures has been reported by Duru and Cayan (2015).

17.4 Biological and Pharmacological Activity of Dietary Diterpenoids and Their Related Compounds

A great number of flower plants including terpenoids have been used as foods, spices, vegetables, and beverages (tea, coffee, juices). Each crude extract including some diterpenes possesses a number of different biological and pharmacological activities. The most frequently recognized activities of diterpenoids are antimicrobial, antifungal, anti-inflammatory, antioxidant, and cytotoxic effects as seen in monoterpenoids (▶ Chap. 16, "Dietary Monoterpenoids").

As shown in Table 1, dietary foods and beverages contain a great number of highly oxygenated diterpenoids possessing γ-lactone and furan rings and almost all of them possess some kind of bio- and pharmacological activity, especially cytotoxic, antimicrobial, antifungal, antioxidant, and anti-inflammatory activity.

Cafestol (**7**) which was obtained from unfiltered coffees indicates hepatoprotective and a potent cholesterol elevating effect in mice (Van Cruchten et al. 2010). In India the major medicinal spices of *Coleus* is the tuberous *C. folskohlii, C. amboinicus, C. blumei, C. tuberosus, C. malabaricus*, and *C. scutellaroides* among which *C. forskohlii* is widely used in different counties. Forskolin (=7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one) (**10**) and its analogues included in *C. folskohlii* has been used for the treatment of heart diseases, abdominal colic, respiratory disorders, and many others and they have been totally synthesized by many organic chemists and their pharmaceutical activities tested. On the other hand, *Coleus carnosus*, which has been used as foods and spice, elaborated abietane diterpenoids, possessing antibacterial and anti-HIV, antiseptic, and potent cytotoxic activity. Kavitha et al. (2010) summarized the history, morphology, phytochemistry, and pharmacological aspects of *C. folskohlii* and forskolin and its analogues in detail. Asakawa et al. (2013) reported the hemisynthesis of forskolin (**10**) and 1,9-dideoxyforskolin (**11b**) from the labdane diterpene, ptychantin A (**10a**) (Fig. 3).

Many species belonging to the Zingiberaceae are rich sources of labdanes of which (*E*)-labda-8(17),12-dien-15,16-dial (**29**) isolated from *Alpinia* and *Curcuma* species which have been used as foods, spices, and beverages shows potent anti-inflammatory and antimicrobial activity. *Andrographis paniculata,* king of bitters, which biosynthesized a number of andorgrapholides, exhibits potent cytotoxic,

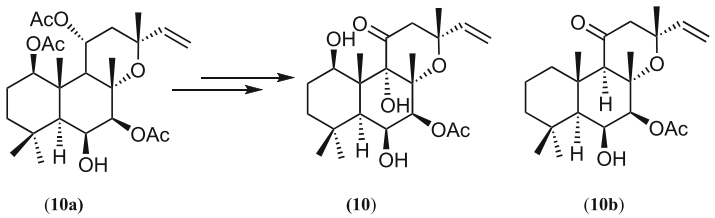


Fig. 3 The preparation of forskolin (**10**) and 1,9-dideoxyforskolin (**10b**) from the labdane diterpene, ptychanitin A (**10a**) isolated from the liverwort, *Ptychanthus striatus* (Asakawa et al. 2013)

cardiovascular, hepatorenal protective, and psychopharmacological activity. The *ent*-kauranes and *ent*-norkarurane-containing *Annona glabra* and *A. squamosal* (sugar apple) indicate antifungal, anti-HIV, cardiotonic activity, antidiabetic, and hypoglycemic, respectively. *Azadirachta indica*, the neem tree, which contains various podocarpa diterpenoids, possesses antidiabetic, antimicrobial, antifungal, and causes abortion, and used for cardiovascular, fever, liver, and skin disorders.

Baccharis species that have been used as beverages are rich sources of clerodane and neoclerodanes. For example, *B. genistelloides* possessing 15,16-epoxy-*ent*-clerodanes and *ent*-cleroda-18,19-olides exhibits antiviral, activity against HIV-1/VSV, antimutagenic, anti-inflammatory, and antidiabetic and have effects on bile duct, bone marrow, and uses for analgesic, anemia, and diarrhea, and so on. There are many *Croton* species beneficial for human health and they mainly produce clerodanes, and some of them are also rich sources of kauranes and labdanes, For example, *C. haumanianus* indicates aphrodisiac, antiepileptic, antihypertensive, and antidiabetic.

Curcuma species belonging to the Zingiberaceae family have been used as foods, foods additives, spices, beverages, and processed foods world widely. They are very rich of sesqui- and diterpenoids and pigment, like curcuminoids. Especially, *C. amada* (mango ginger) which produces a number of labdanes indicates various pharmacological activity, such as cytotoxicity against many cancer cell lines, anti-platelet, antifungal, and antimicrobial effects.

Ginkgo biloba and *Zingiber officinale* are very important crude drugs and ginkgolides (**11**–**13**) indicates anti-Alzheimer activity.

Dioscorea bulbifera, a Traditional Chinese Medicine (TCM) and food, contains several clerodane diolides, such as cleroda-16, 16β:18,2α-diolide (=diosbilbin B), which demonstrates cytotoxic activity against several cancer cell lines, fungi, and bacteria.

Eremophila species are chemically very characteristic since their major components are serrulatane type diterpenoids indicating α-glucosidase inhibitory activity as shown in *E.* aff. *drummondii*, *E. falcata*, and *E. glabra*.

The species belonging to the *Euphorbia* biosynthesize a number of different types of diterpenoids, and some species, such as *E. fischeriana*, elaborate highly oxidized phorbol esters which show antiviral activity against HIV-1 and rosane and abietane types possessing potent cytotoxic against human breast, colon, and prostate cancer cells. *E. helioscopisa* used as tea substitute contains jorkinane type diterpenoids indicating antiasthmatic, anthelmintic, antifungal, antimicrobial, and cytotoxic against lung cancer, and NO production inhibitory activity. *E royleana* elaborates various types of diterpenoids, euphanes, ingenols, kauranes, abietanes, and isopimaranes, possessing cytotoxicity against several human cancer cells.

The presence of diterpenoids in mushrooms is very rare. *Hericium* species are exceptional, since they produce many cyatane diterpenoids which show nerve growth factor synthesis stimulatory activity.

A few *Icacina* species, such as *I. trichantha* which has been used as food, produces various icacinane diterpenoids having antiasthma, antihypertensive, antimicrobial, and antioxidant.

The *Isodon* species are very rich sources of *ent*-kauranes. Up to now, more than 300 of this type of compounds have been isolated, and many of them indicate various biological activity. *I. japonica* used as beverage contains enmein (**15**), nodosin, isodocarpin, and their related compounds which indicate cytotoxic effect, murine lymphoma and antioxidant and antimicrobial activity. *Lycopus europaeus* is a rich source of isopimaranes possessing antifungal, antigonadotropic, antimicrobial, and antioxidant.

Oryza sativa (rice) produces a few characteristic pimaranes, such as momilactone A and its analogues as well as various gibberellins which inhibit germination inhibitory activity.

Rubsoside (**19**) and stevioside (**20**) isolated from *Rubus suavissimum* are significant kaurene-type sweet substances. They are widely used as food additives and sweeteners for many beverages and cooked foods and many other processed foods (Kinghorn et al. 2010). *Salvia* are rich sources of diterpenoids which show a number of different types of bio- and pharmacological activity. Tanshinone (**21**), and its analogues from *Saliva miltiorrhiza* show apoptosis in human breast cancer and are anti-inflammatory.

Salvia rosmarinus (=*Rosmarinus officinalis*) which has been used as flavoring food, spice, cosmetics, beverage, and crude drug produces phenolic abietanes, rosmanol (**17**), isorosmanol (**18**), and carnosic acid. Rosmanol has anti-inflammatory, antioxidant, antidepressant, and significant cytotoxic activity against the neuroblastoma and human colon adenocarcinoma cells, and antiulcerogenic activities. Isorosmanol (**18**) also exhibits antioxidant, neuroprotective, and neurotrophic effects and acetylcholinesterase inhibitory activity. Carnosic acid is more efficient antioxidant than vitamin E. *Salvia officinalis* used as foods, food flavor, crude drugs, and spice elaborates the same abietanes lactones (**17, 18**) from *S. rosmarinus*, and many other abietanes show many significant pharmacological activity, antimicrobial, antifungal, antispasmodic, antioxidant as well as cytotoxic, hemostatic, and hypoglycemic activity.

Many *Stachys* species have been used as beverages (herbal tea). *Stachys* species produces a number of different diterpenoids. *S. palustris* which elaborates abietanes and *ent*-kauranes indicates antibacterial, antifungal, antioxidant, antispasmodic, and antiseptic activity.

Stevioside (**20**) is the major *ent*-kaurane glucoside from *Stevia rebaudiana* together with its various analogues (**20a–f**) as shown in Chart 5 (Kinghorn et al. 2010). Stevioside is one of the most significant food additive (sweetener) for various kinds of foods, especially for non-calorie sweetener for beverage (coffee), cakes, cookies, and sources.

Since Wani et al. (1971) isolated paclitaxel (=taxol), one of the most significant clinically used anticancer drugs, from *Taxus brevifolia*, its total synthesis, isolation, and structural elucidation as well as biodegradation and biotransformation have been carried out and more than 200 taxane products have been obtained in the last century.

Teucrium chamaedrys (Germander), *T. montanum, T. pilum*, and *T. scorodonia* which mainly produce clerodanes and neo-clerodane lactones have been used for beverage (herbal tea, liqueurs, and flavored wine). These diterpenoids indicate antibacterial, antioxidant, diuretic, and hepatotoxic effects. *Vitex rotundifolia*,

which biosynthesizes a number of highly oxygenated labdanes, show anti-hyperlipidemic and antiprasmodial activity.

Cembrane-type diterpenoids are a large and structurally varied group of natural products isolated from both terrestrial and marine organisms. Especially, the Greek tobacco elaborates different types of cembranes many of which contain a γ-lactone group (Connolly and Hill 1991). Interestingly, some liverworts produce highly oxygenated cembranes (Asakawa et al. 2013). From a biomedical perspective, cytotoxicity is the most remarkable property of this class of diterpenoids, represented by sarcophytol originated from soft coral (Connolly and Hill 1991).

17.5 Microbial and Mammalian Biotransformation of Diterpenoids and Their Related Compounds

Every day, we take consciously take foods, vegetables, fruits, processed foods, spices, beverages (tea and herbal tea, coffee, cocoa) as well as processed foods including secondary metabolites, terpenoids, steroids, aromatic compounds and acetogenins, alkaloids, and so on. Little attention has been payed to fetal (metabolic pathways) of these compounds in our body, although many secondary metabolites were biotransformed by microorganisms and enzymatic treatment to produce the different functional compounds than the original products.

Recently, a numerous diterpene synthase and cytochrome P450 enzymes which generate different diterpenoids have been identified. Such enzymatic catalysts and their profound knowledge of specialized terpenoid metabolism may be applied to modern microbial and photosynthetic production systems which offer alternative production of different type of phytochemicals for human health (Mafu and Zerbe 2018).

A number of the papers concerning bioconversion of terpenoids, especially mono-, sesqui-, and diterpenoids, have been reported (Asakawa and Noma 2010, 2020; Bhatti and Khera 2014; Frij et al. 2011; Monteiro et al. 2017; Noma and Asakawa 2020; Rajamanikyam et al. 2017; Silva et al. 2013; Sultana and Saify 2012). Abietanes, cembrane, clerodanes, kauranes, labdanes, pimaranes, and taxanes and some minor skeletal diterpenoids have been carried out by enzymatical and in situ biotransformation in mammals in order to know the fetal (pathway of substrate) in our body and to obtain some functional substances for application to cosmetics, medicinal, and agricultural drugs as well as food additives and beverages.

In the following figures, the biotransformation pathways of some representative dietary diterpenoids have been shown.

17.5.1 Abietanes

Rodents, especially wild hares, like to eat conifer plants including diterpenoids, such as abietane and its derivatives, and kaur-16-ene. In order to understand the fetal of such simple diterpenoids, abietic acid (**2**) was orally administered directly into rabbits for 24 h, followed by the enzymatic degradation of urine to give C-16

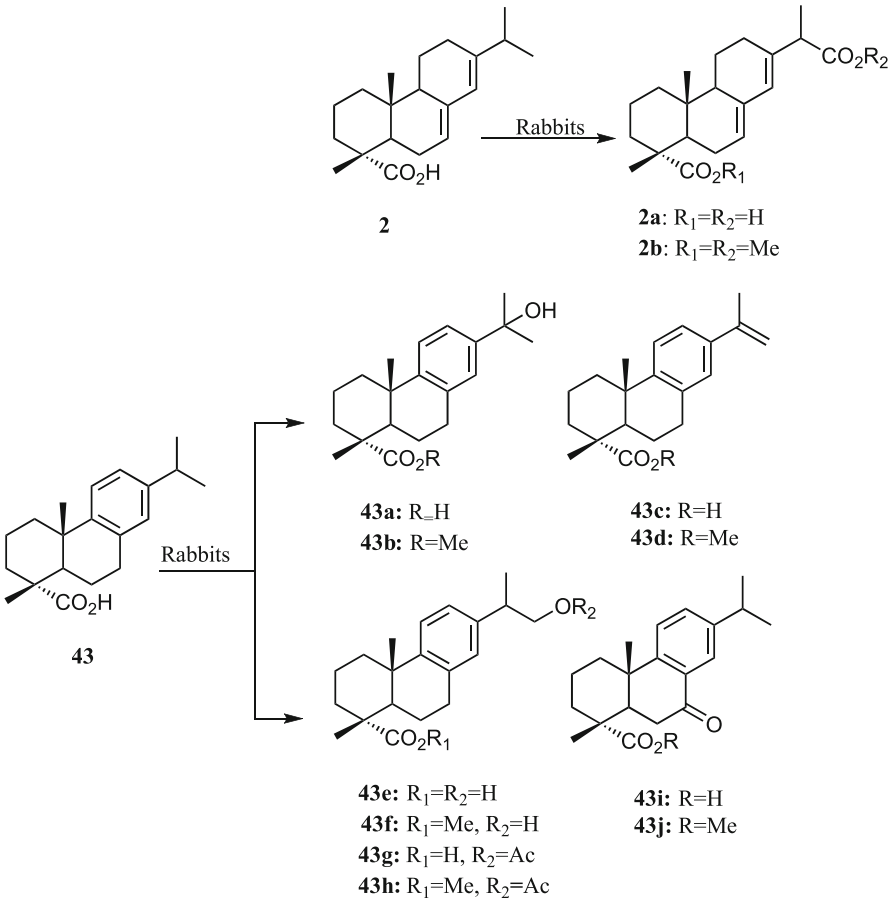


Fig. 4 Biotransformation of abietic acid (**2**) and dehydroabietic acid (**43**) by rabbits (Asakawa and Noma 2010)

carboxylic acid (**2a**) (Asakawa and Noma 2010, 2020) (Fig. 4). Dehydroabietic acid (**43**) was treated in the same manner as described above to give the metabolites (**43a–d**). Hydroxylation at an isopropyl group and oxidation at C-7 position and dehydration of the metabolic product (**43b**) were found in this case. 8,11,13-Abietatrien-18-ol (**44**) was treated in the same method as mentioned above to form seven metabolites (**44a–g**). Oxidation and hydroxylation at C-2 were observed together with the same hydroxylation at an isopropyl group seen in dehydroabietic acid (Fig. 5) (Asakawa and Noma 2010, 2020).

Cryptotanshinone (**45**) possessing an orthquinone group from *Salvia* species was treated by two microorganisms, *Cunninghamella elegans* or *Mucor rouxii.* The former fungous directly introduced stereo- and regiospecifically a hydroxyl

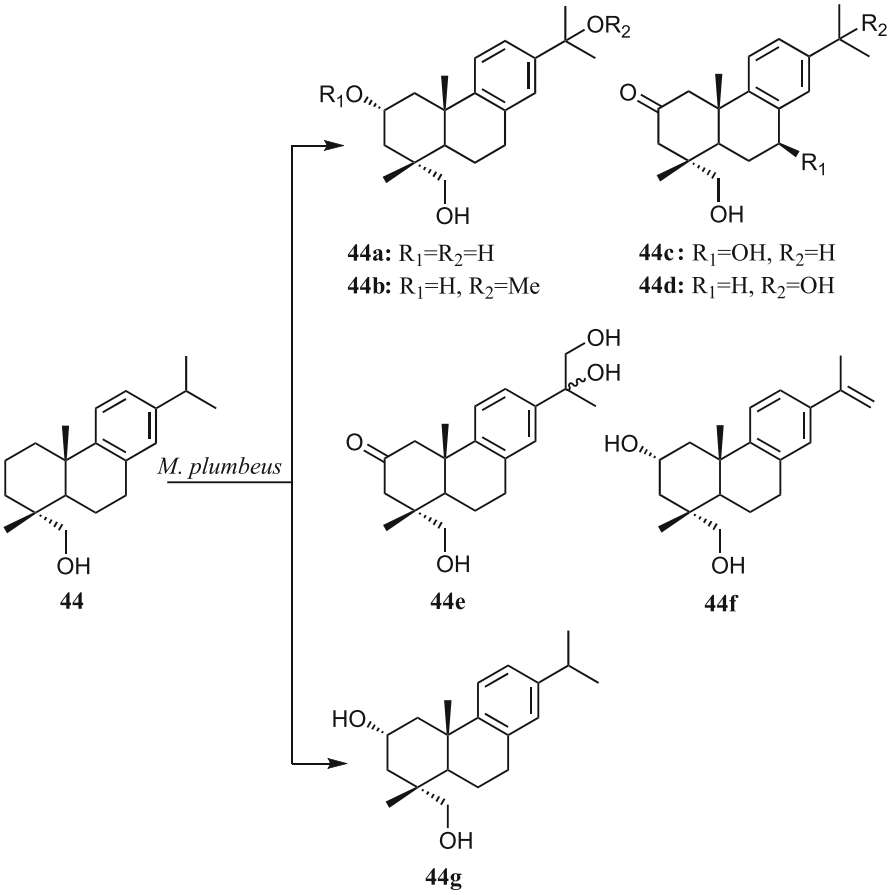


Fig. 5 Biotransformation of 8,11,13-abietatrien-18-ol (**44**) by *Mucor plumbeus* (Asakawa and Noma 2010)

group at C-3 and C-18 position to give 3α- (**45a**), and 3β- (**45b**) and 18-hydroxydated cryptotanshinone (**45c**) (Martinez et al. 2014) and the latter microorganism gave totally different metabolites than those obtained from the same substrate mentioned above. It produced three anhydrides (**45d–f**) and four spiro lactones (**45g–j**), two of which have 3α- (**45g**) and 3β-hydroxyl group (**45h**) (Fig. 6) (De Sousa et al. 2018).

17.5.2 Ginkgolide B

Ginkgolide B (**12**) obtained from *Ginkgo biloba* exhibits significant pharmacological effects against ischemic brain injury, platelet aggregation, and thrombosis as

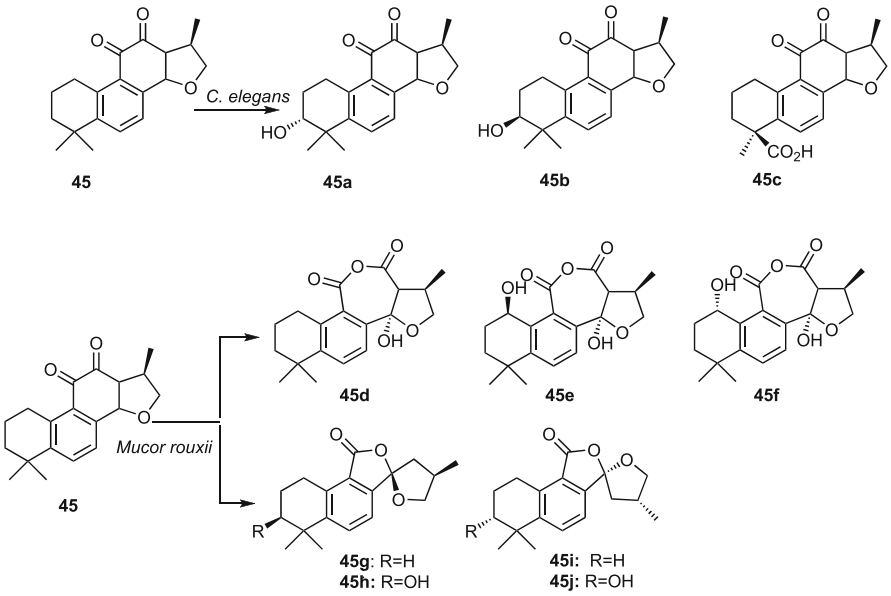


Fig. 6 Biotransformation of cryptotanshinone (**45**) by *Cunninghamell elegans* (De Sousa et al. 2018) and *Mucor rouxii* (Martinez et al. 2014)

well as inflammatory and central nervous disorders, post-ischemic neuronal damage, dementia, and Alzheimer's disease. Wang et al. (2008) administered ginkgolide B (**12)** to rats in order to understand its metabolite pathway. The rat biotransformed to ginkgolide B to a hydroxy ginkgolide B (**12a**) and an A-ring hydroxylated ginkgolide B monool **(12c**) via **12b**. This conversion was confirmed by rat liver incubation in vitro and the CYP2D6 isozyme was identified as the major CYP450 enzyme responsible for ginkgolide B metabolism in the rat liver microsomes (Fig. 7).

17.5.3 Cembranes

Since discovery of cembrane type diterpenoids in marine organisms, especially from soft corals, a number of cembranoids have been found not only in marine animals and algae but also in higher plants mainly from some tobacco species and liverworts. A cembrene diol, 2,7,11-cembratriene-4α,6α-diol (**46**) was converted by two microorganisms, *Cunninghamella elegans* and *Mucor ramannianus* to afford 11,12-epoxy-6α-acetate (**46a**). 4α-Hydroxy-6α-acetoxy-2,7,11-cembratriene (**46b**) was biotransformed by *Bacillus megaterium* to furnish a cembrene triol (**46c**) (Fig. 8) (Asakawa and Noma 2010, 2020).

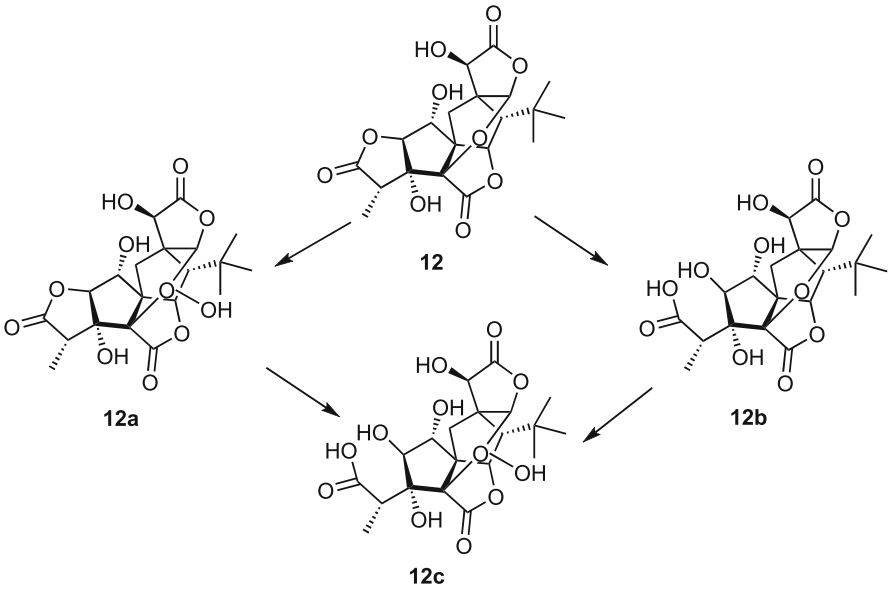


Fig. 7 Biotransformation of ginkgolide B (**12**) in rat (Wang et al. 2008)

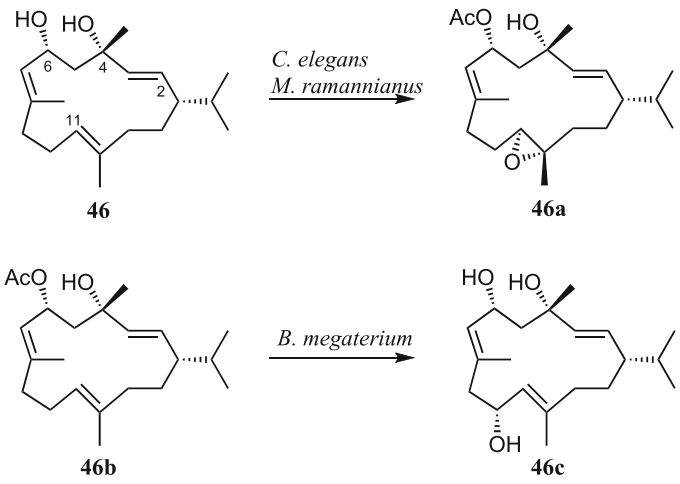


Fig. 8 Biotransformation of 2,7,11-cembratriene-4,6-diol (**46**) and 6-acetoxy-2,7,11-cembratrien-4-ol (**46b**) by *Cunninghamella elegans*, *Mucor ramannianus*, and *Bacillus megaterium* (Asakawa and Noma 2010)

17.5.4 Kauranes

Cafestol (**7**) which is one of a furano-kaurane 16,17-diol, from coffee extract was biotransformed to 2-hydroxycafestol (**7a**), followed by epoxidation and successive epoxidation on one of the double bond of a furan ring to give an epoxide (**7b**) and then to glutathione conjugate (**7b**) by liver P450 enzyme (Fig. 9) (Van Cruchten et al. 2010). When a sweet dietary diterpene glucoside, stevioside (**20**) was orally taken in human body, it was converted to steviol glucuronide (= steviol 19-*O*-β-glucopyranosiduronic acid) (**24a**) in human urine via steviol (**24**) (Geuns et al. 2006). On the other hand, some microorganisms, *A. niger*, *M. recurvatus*, and *B. emgaterium* converted steviol (**24**) to *ent*-7α-hydroxy- (**24b**), *ent*-19-oic acid β-glucopyranosyl ester (**24c**), *ent*-16β,17-dihydroxy- (**24d**), 7-oxo- (**24e**), and 7α,11β-dihydroxysteviols (**24f**) (Fig. 10) (Asakawa and Noma 2010, 2020). *ent*-Kaur-16-en-19-oic acid (**47**) which is one of the representative kauranes widely distributed in dietary foods and medicinal plants was incubated by *C. blakesleeana* to give four metabolites, *ent*-7α- (**47a**), *ent*-16α-hydroxy- (**47b**), *ent*-16β,17-dihydroxy- (**47c**), and *ent*-7α,16β-dihydroxykauranes (**47d**) (Fig. 11). *Ent*-kaur-16-en-7β-ol (**48**) was treated in the same manner as mentioned above by *Gibberella fujikuroii* to furnish *ent*-7β,16β,17-triol (**48a**) and unique secokaurene anhydride (**48b**). Further biotransformation of *ent*-7β,18-dihydroxykaur-16-ene (**49**) and *ent*-7β,15β,18-trihydroxykaur-16-ene (**50**) by *G. fujikuroii* gave *ent*-7β,18,19-trihydroxykaurene (**49a**) and *ent*-7β,11α,15β,18-tetrahydroxykaur-16-ene (**50a**), respectively (Fig. 12) (Asakawa and Noma 2010, 2020).

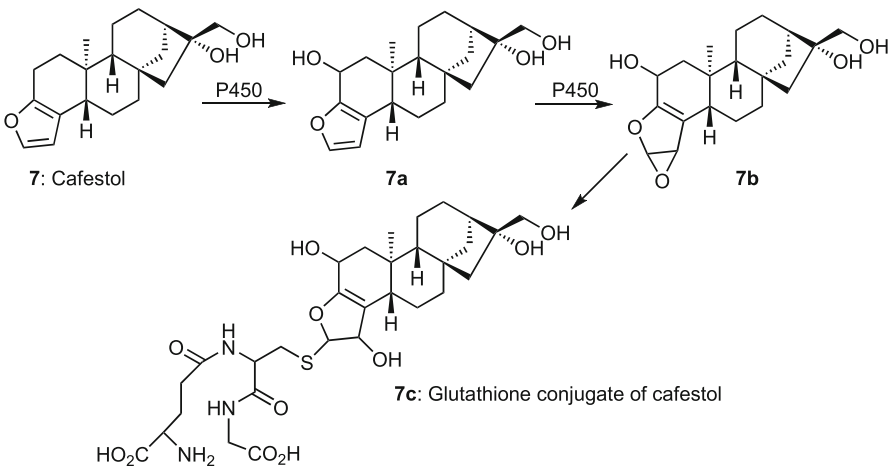


Fig. 9 Biotransformation of cafestol (**7**), *ent*-kaurane-type diterpenoids in mice liver P-450 (Van Cruchten et al. 2010)

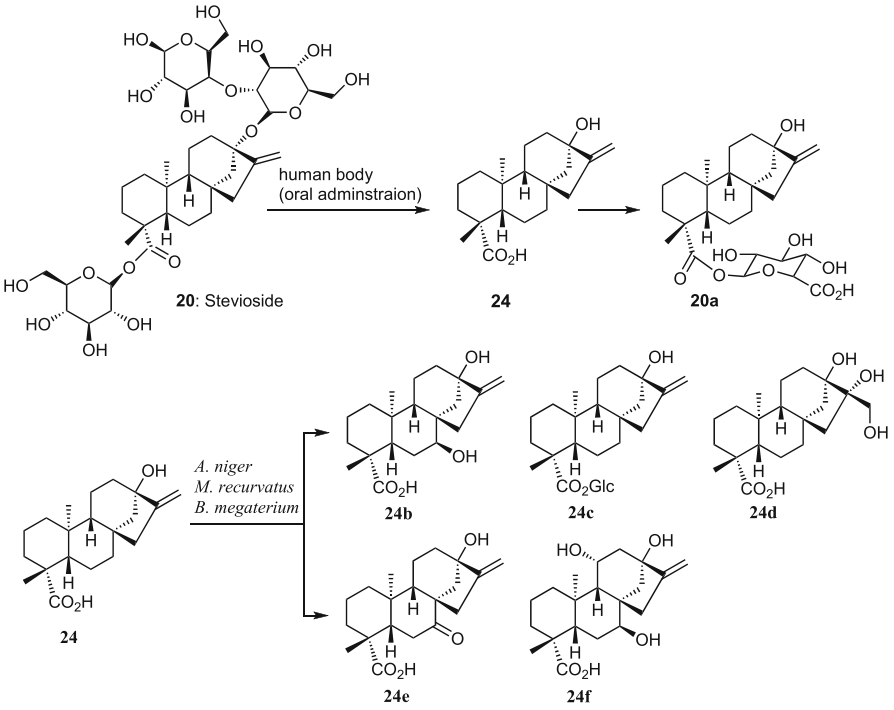


Fig. 10 Biotransformation of stevioside (**20**) by human body (Geuns et al. 2006) and steviol (**24**) by *Aspergillus niger*, *Mucro recurvatus*, and *Bacillus megaterium* (Asakawa and Noma 2010)

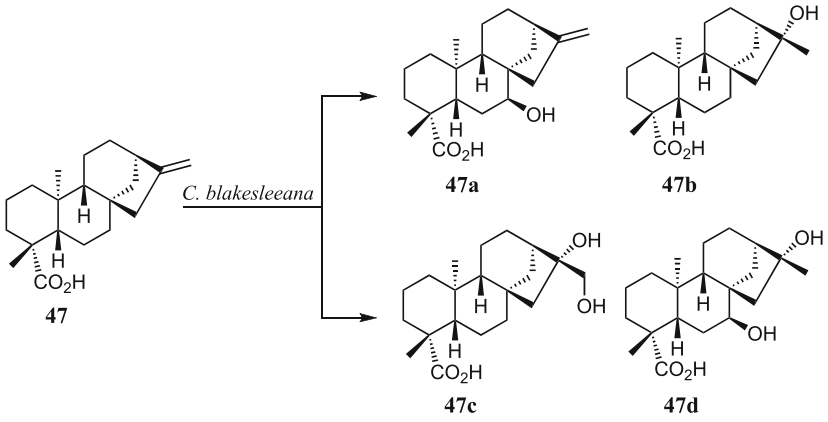


Fig. 11 Biotransformation of *ent*-kaur-16-en-19-oic acid (**47**) by *Cunninghamella blakesleeana* (Asakawa and Noma 2010)

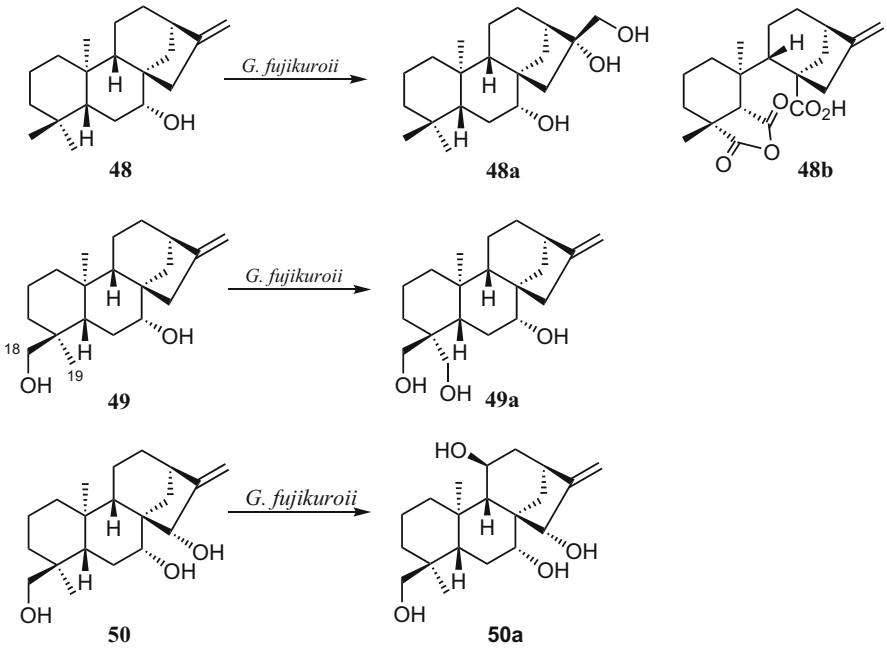


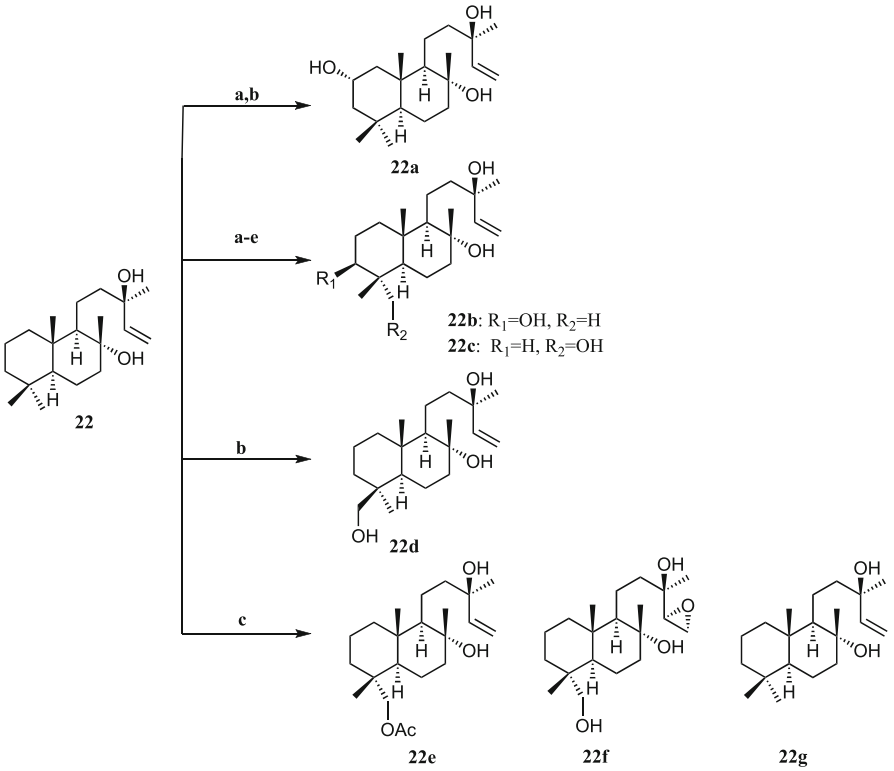
Fig. 12 Biotransformation of *ent*-kaur-16-en-7β-ol (**48**), *ent*-7β,18-dihydroxy-kaur-16-ene (**49**), and *ent*-7β,15β-trihydroxy-kaur-16-ene (**50**) by *Gibberella fujikuroi* (Asakawa and Noma 2010)

17.5.5 Labdanes

Sclareol (**22**), a labdane type diol, from some *Salvia* species was biotransformed by four microorganisms as shown in Fig. 13. Each microorganism regio- and stereo-selectively gave 2α-hydroxy- (**22a**), 3β-hydroxy- (**22b**), 19-hydroxy- (**22c**), 18-hydroxy- (**22d**), 19-acetoxy- (**22e**), and 15,16-epoxy product (**22f**) of 19-hydroxy compound (**22c**) (Asakawa and Noma 2010, 2020). Manool (**34**) and 7α-hydroxymanool (**51**) were also treated by *Mucor plumbeus* to afford the similar hydroxylated products (**34a–d**) and **51a–c**, respectively, as those from sclareol (**22**) (Fig. 13) (Asakawa 2010; ► Chap. 16, "Dietary Monoterpenoids").

17.5.6 Taxanes

In order to obtain paclitaxel (=taxol) like more potent and safe anticancer reagents, a number of biotransformation experiments of taxoids have been carried out. The biotransformation of paclitaxel (=taxol) (**1**), cephalomannine (**52**), and baccatin III (**53**) by *Nocardioides albus* is shown in Fig. 14. Firstly hydrolysis of **1** at C-13 occurred to afford baccatin-III, followed by hydrolysis at C-10 to furnish



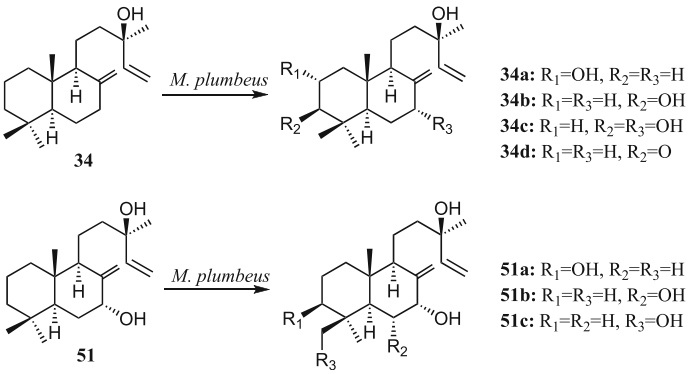


Fig. 13 Biotransformation of sclareol (**22**), manool (**34**), and 7α-hydroxymanool (**51**) by various microorganisms (Asakawa and Noma 2010)

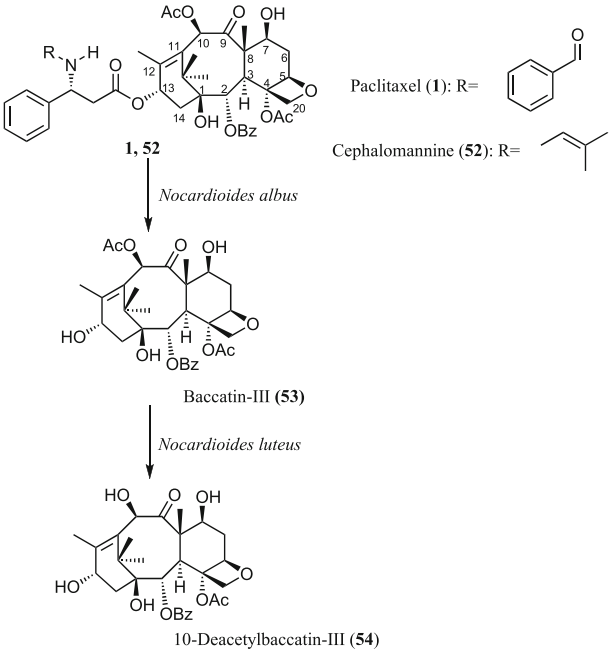


Fig. 14 Biotransformation of paclitaxel (= taxol) (**1**), cephalomannine (**52**), and baccatin III (**53**) by *Nocardioides leteus* (Asakawa and Noma 2010)

10-deacetylbaccatin III (**53a**). 10-Deacetyl-7-epi-taxol (**54**) was treated with two microorganisms, *Microsphaeropsis onychiuni* and *Mucor* species gave 10-desacetylpaclitaxel (**53a**) and two C-10 hydrolyzed products (**54b, c**) (Fig. 15).

Sinenxan (taxuyunnanine C) (**55**) was incubated with *Abisidia coerulea* to afford C-10 deacetyl (**55a**) and four hydroxylated products (**55b-e**) at B- and C-rings, respectively (Fig. 15) (Asakawa and Noma 2010, 2020).

17.5.7 Clerodanes

A furanoclerodane lactone (**56**) isolated from *Dodonea viscosa* was incubated with a plant pathogen, *Rhizopus stolonifera* to afford a furan-ring cleaved lactone (**56a**) and *ent*-2β-hydroxy introduced product (**56b**), while a furanocleroda-19-oic acid methyl ester (**57**) was treated in the same manner as that described above to furnish two γ-lactones (**57a, c**) and two γ-lactone cleaved metabolites (**57b, d**). Among all of the metabolite, 15-hydroxy-13-oic acid (**57d**) indicated antimicrobial activity against Gram negative bacteria (Fig. 16) (Choudhary et al. 2013).

17.5.8 Pimaranes

When *ent*-13-epi-Pimara-9(11),15-dien-19-oic acid (**58**) was fed with the fungous *Gibberella fujikuroii*, *ent*-1-oxo-2β, 9β-dihydroxy- (**58a**) and its C-2-epimer (**58b**),

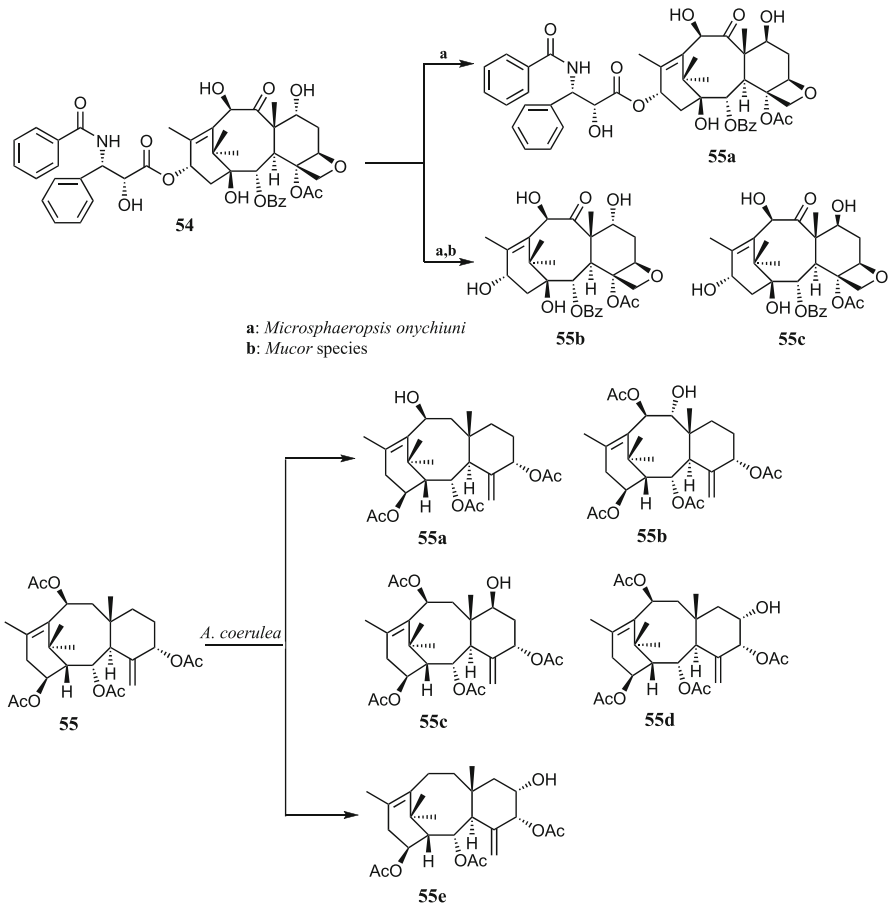


Fig. 15 Biotransformation of 10-deacetyl-7-epi-taxol (**54**) by *Microsphaeropsis onychiuni* and *Mucor* species, and Sinenxan **(55)** by *Abisidia coerulea*

1-oxo-12β-hydroxy derivative (**58c**) were obtained together with a Baeyer-Villiger oxidative product (**58d**). On the other hand, two *ent*-8β-hydroxy-9,11-epoxy derivatives (**59a, d**) were obtained along with two *ent*-7-oxo-11β derivative (**59b, c**) were obtained from *ent*-13-epi-pimara-9(11),15-dien-19-ol (**59**) (Fig. 17) (Martinez et al. 2014).

17.5.9 Fusicoccanes

Hypoestes forscalei is a rich source of fusicoccane diterpenoids. Al Haidari (2018) reported the biotransformation of hypoestenone (**59d**) by fungous, *Mucor ramannianus* ATCC 9628. An epoxidation occurred in this case to give 8α,9-α-epoxyhypoestenone (**59e**) (Fig. 18).

Only several examples of enzymatical biotransformation of some diterpenoids by several fungi and rabbits have been demonstrated. A number of diterpenoids were

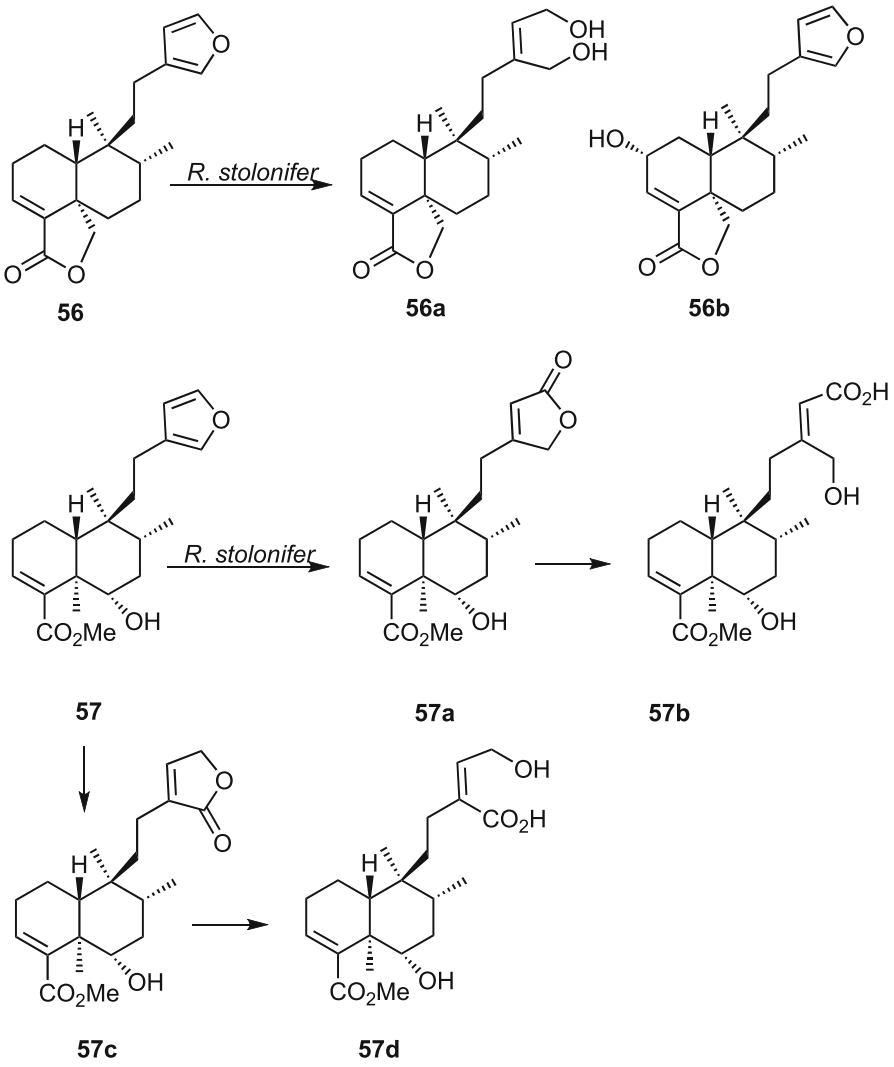


Fig. 16 Biotransformation of clerodane lactone (**56**) and clerodane methyl ester (**57**) isolated from *Dodonaea viscosa* and *Pulicaria wightiana*, respectively, by a plant pathogen fungus *Rhizopus stolonifera*

biotransformed by various fungi and mammals to give various metabolites, many of which showed antimicrobial, antifungal antiobesity, cytotoxic, and enzyme inhibitory activity. In the microbial and mammalian biotransformation, oxidation (direct introduction of hydroxy and carbonyl group at non-active carbon atom and carboxylation), reduction (double bond and ketone), epoxidation (double bond), esterification (including lactonization), and glycosidation have been found. The present methods are environmentally very friendly because a substrate is soluble in water with some nutrients, like peptone, simple one step reaction, non-hazard, and very

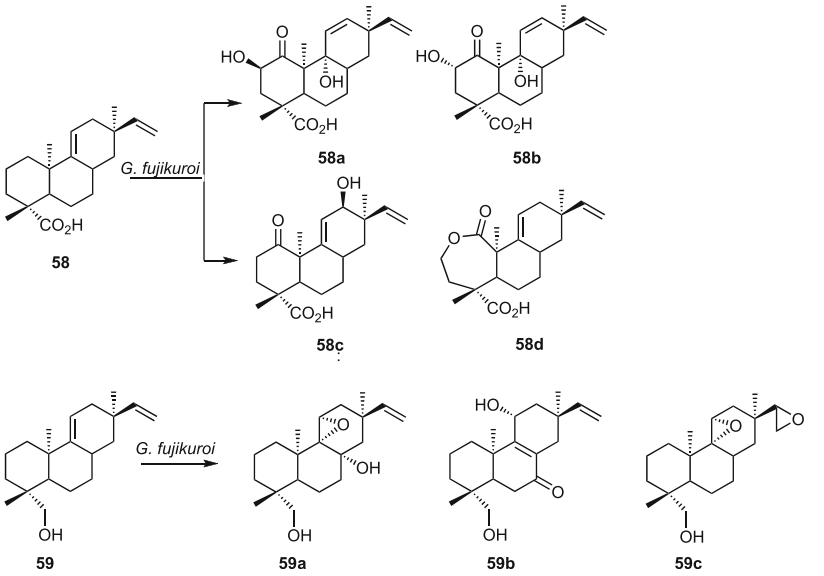
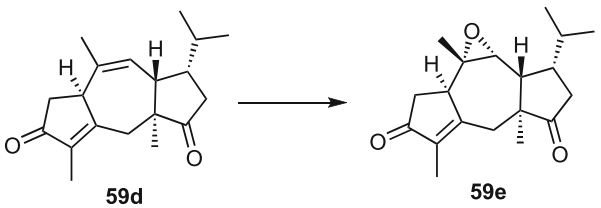


Fig. 17 Biotransformation of 13-epi-*ent*-pimara-9(11),15-dien-19-oic acid (**58**) and its 19-ol (**59**) by *Gibberella fujikuroi* (Martinez et al. 2014)

Fig. 18 Biotransformation of hypoestenone (**59d**) by *Mucor ramannianus* (Al Haidari 2018)



cheap, and it gives many valuable metabolites possessing different bio- and pharmacological activity from freely selected dietary diterpenoids and those from many medicinal and aromatic plants.

17.6 Conclusion

Three major dietary diterpenoids found in coffee, *Ginkgo*, and *Stevia* species and many other medicinal plants and one animal originated aroma, ambrox, have been discussed from viewpoint of foods, beverages, foods additives, and supplements as well as their bio- and pharmacological activity. Furthermore, the biotransformation of dietary diterpenoids including coffee, *Ginkgo biloba*, *Salvia*, *Taxus*, etc. and the other specific diterpenoids using enzymes and in situ mammals, such as rabbits was demonstrated in detail. In this chapter, more than 900 higher plants and 50 spore-forming plants (ferns, bryophytes, algae, lichens) and some mushrooms species

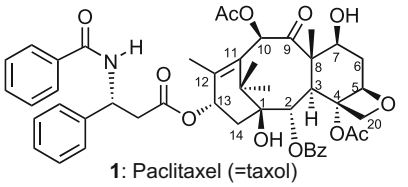
producing diterpenoids have been given to fields of foods, beverages, food additives, and medicinal sources. So many diterpenoids were tested against cancer cell lines, harmful microorganisms such as bacteria, viruses, and fungi. One of the most significant discoveries of anticancer drug from plant is taxol from *Taxus brevifolia* and *T. canadensis*. In order to find more active anticancer drugs, many taxane compounds have been biotransformed using tissue culture and bioconversion technique and hemisynthesis. A great amount of stevioside, non-calorie sweetener have been consumed in the world without any serious side effect, and *Ginkgo* extract containing ginkgolides having a unique tertiary butyl group in the molecule has been used as the effective drug for Alzheimer's diseases. It is absolutely difficult for the authors to get all of dietary diterpenoids references from the world. The authors think that a number of significant foods, vegetables, beverages, food additives, and processed foods including diterpenoids using in each country are lack in Table 1; however, the authors believe that Table 1 could be used as an Encyclopedia to understand how each plant and fungous, but not many animals (used as foods, vegetables, tea and coffee, and alcoholic beverages), biosynthesizes so many different types of diterpenoids, several of which exhibit incredible bitter and sweet tastes. Recent computer's development, analytical techniques, molecular biology, and omics studies will create more significant and valuable nutrients, medicinal drugs as well as food additives, supplement, and cosmetics for our humane life.

17.7 Structure Numbers and Names of Diterpenoids Including This Chapter

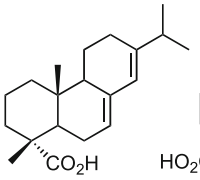
(**1**) Paclitaxel (= taxol)
(**2**) Abietic acid
(**3**) (*E*)-Communic acid
(**4**) 16β,17-Dihydroxy-*ent*-kauran-19-oic acid
(**5**) *ent*-11α-Hydroxy-16-kauren-15-one
(**6**) Labda-7,13*E*-diene-3β,15-diol
(**7**) Cafestol
(**8**) 16-*O*-methylcafestol
(**9**) Kahweol
(**10**) Forskolin
(**11**) Ginkgolide A
(**12**) Ginkgolide B
(**13**) Ginkgolide C
(**14**) Nagilactone A
(**15**) Enmein
(**16**) Carnosol
(**17**) Rosmanol
(**18**) Isorosmanol
(**19**) Rubsoside
(**19a**) Suavioside A
(**19b**) Steviol monoside

(**19c**) Suavioside B
(**19d**) Suavioside G
(**19e**) Suavioside J
(**19f**) Suavioside H
(**19g**) Suavioside I
(**19h**) *ent*-3-Oxo-16β-hydroxykaurane-17-*O*-β-glucoside
(**19i**) Cussoracoside A
(**20**) Stevioside
(**20a**) Steviolbioside
(**21**) Tanshinone I
(**22**) Sclareol
(**23**) Ambrox
(**24**) Steviol
(**25**) Rebaudioside A
(**25a**) Rebaudioside B
(**25b**) Rebaudioside C (=dulcoside B)
(**25c**) Rebaudioside D
(**25d**) Rebaudioside E
(**25e**) Rebaudioside F
(**25f**) Dulcoside A
(**26**) Galanolactone
(**27**) Miogadial
(**28**) Galanal A
(**29**) (*E*)-Labda-8(17),12-diene-15,16-dial
(**30**) Copallic acid
(**31**) 3*E*, 7*E*,18-Dolabellatrien-14-one
(**32**) 1(15),8-Dolastadiene-2β,14β-dial (=isoamijiol)
(**33**) 4,18-Dihydroxy-1(9),6,13-xenicatrien-19-al
(**34**) Manool
(**35**) Sandaracopimaric acid
(**36**) (-)-*ent*-Kauran-16α-ol
(**37**) *ent*-Kauran-16*S*,17-hydroxy-19-oic acid
(**38**) 5β,11β,12β-Trihydroxy-15-oxo-*ent*-kaur-16-en-19-oic acid
(**39**) Erinacine A
(**40**) Erinacine B
(**41**) Erinacine C
(42a) Erinacine P
(42b) Erinacine Q
(**43**) Dehydroabietic acid
(**44**) 8,11,13-Abietatrien-18-ol
(**45**) Cryptotanshinone
(**46a**) 2,7,11-Cembratriene-4,6-diol
(**46b**) 6-Acetoxy-2,7,11-cembratrien-4-ol
(**47**) *ent*-Kaur-16-en-19-oic acid
(**48**) *ent*-Kaur-16-en-7β-ol
(**49**) *ent*-7β,18-Dihydroxykaur-16-ene

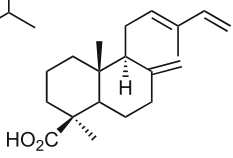
(**50**) *ent*-7β,15β,18-Trihydroxykaur-16-ene
(**51**) 7α-Hydroxymanool
(**52**) Cephalomannine
(**53**) Baccatin-III
(**54**) 10-Deacetyl-7-epi-taxol
(**55**) Sinenxan
(**56**) Furanoclerodane dilactone
(**57**) Furanocleroda-19-oic acid methyl ester
(**58**) *ent*-13-epi-Pimara-9(11),15-dien-19-oic acid
(**59**) *ent*-13-epi-Pimara-9(11),15-dien-19-ol
(59d) Hypoestenone



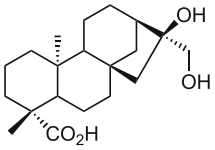
1: Paclitaxel (=taxol)



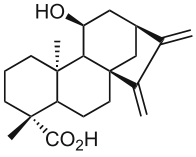
2: abietic acid



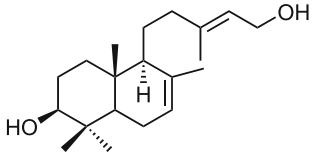
3: (*E*)- communic acid



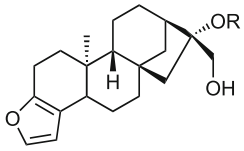
4: 16β-17-dihydroxy-*ent*-kauran-19-oic acid



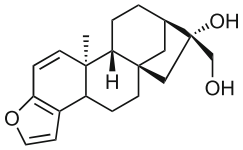
5: *ent*-11α-hydroxykaur-16-en-15-one



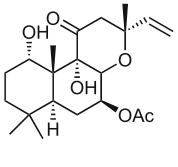
6: labda-7,13*E*-diene-3β,15-diol



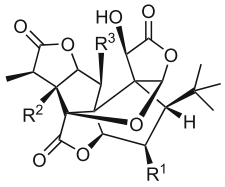
7: R= H: cafestol
8: R=Me: 16-*O*-methylcafestol



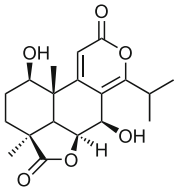
9: kahweol



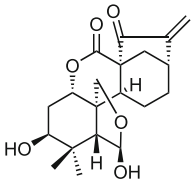
10: forskolin



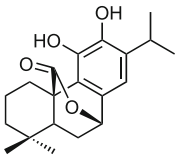
11: ginkgolide A R^1=R^3=H, R^2=OH
12: ginkgolide B R^1=H, R^2=R^3=OH
13: ginkgolide C R^1= R^2=R^3=OH



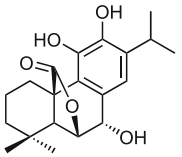
14 : nagilactone A



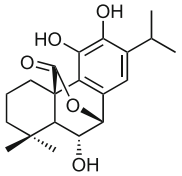
15: enmein



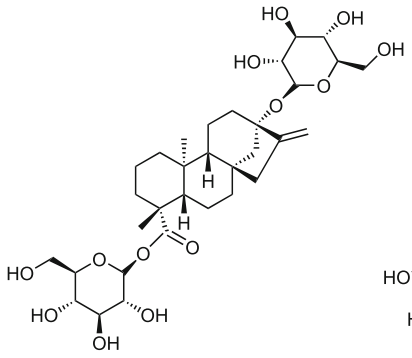
16: carnosol



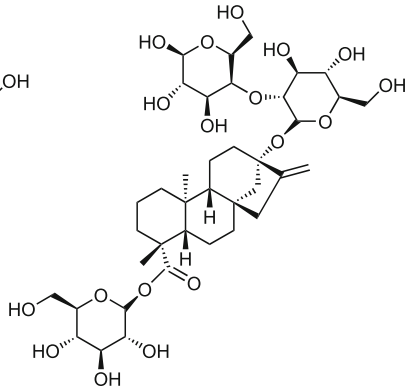
17: rosmanol



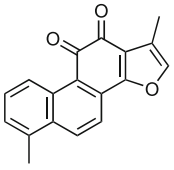
18: isorosmanol



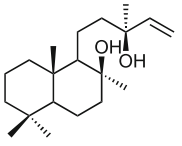
19: rubusoside



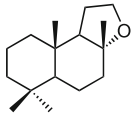
20: stevioside



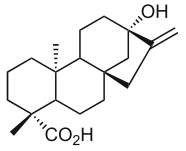
21: tanshinone I



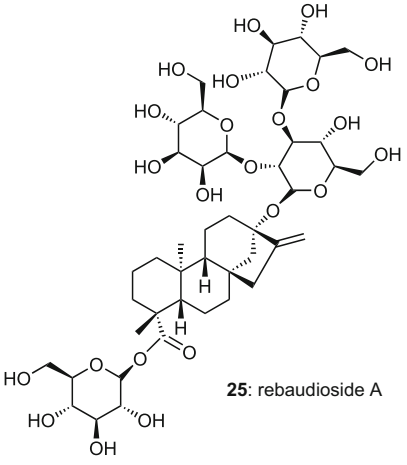
22: sclareol



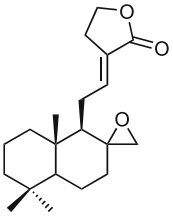
23a: ambrox



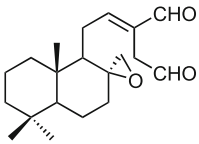
24: steviol



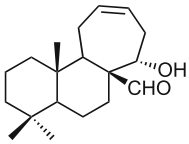
25: rebaudioside A



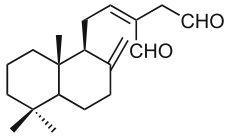
26: galanolactone



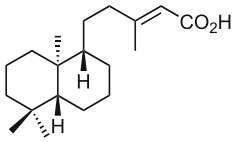
27: miogadial



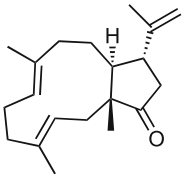
28: galanal A



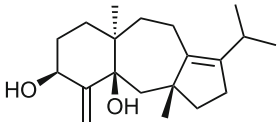
29: (*E*)-Labda-8(17),12-dien-15,16-dial



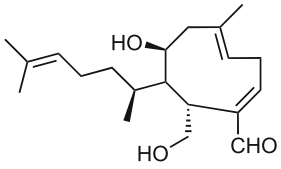
30: copallic acid



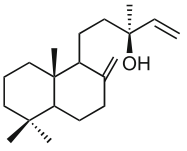
31: 3*E*,7E,18-dolabellatrien-14-one



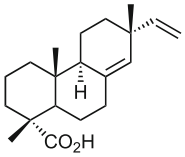
32: 1(15),8-dolastadiene-2β, 14β-diol (=isoamijiol)



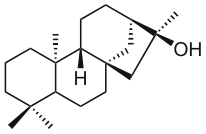
33: 4,18-dihydroxy-1(9),6, 13-xenicatrien-19-al



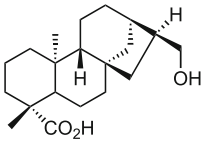
34: manool



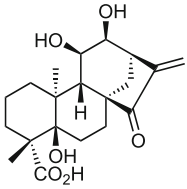
35: sandaracopimaric acid



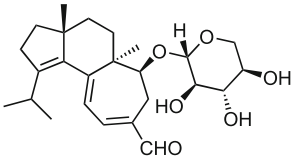
36: *ent*-kauran-16α-ol



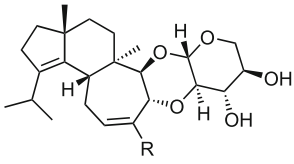
37: *ent*-kauran-16*S*,17-hydroxy-19-oic acid



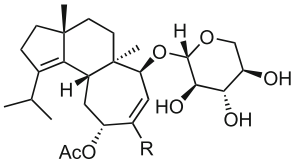
38: 5β,11β,12β-trihydroxy-15-oxo-*ent*-kaur-16-en-19-oic acid



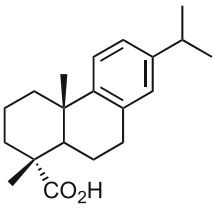
39: erinacine A



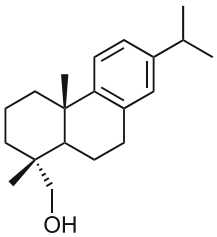
40: erinacine B: R=CHO
41: erinacine C: R=CH_2OH



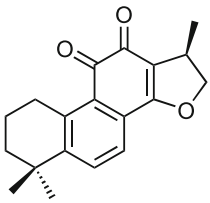
42a: erinacine P: R=CHO
42b: erinacine Q: R=CH_2OH



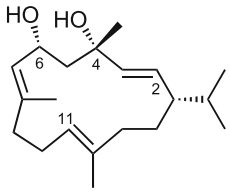
43: dehydroabietic acid



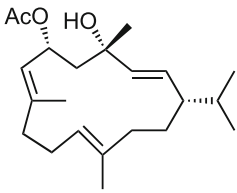
44: 8,11,13-abietatrien-18-ol



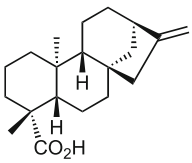
45: cryptotanshinone



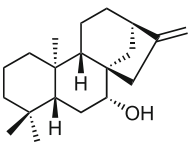
46a: 2,7,11-cembratriene-4,6-diol



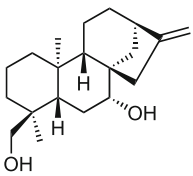
46b: 6-acetoxy-2,7,11-cembratrien-4-ol



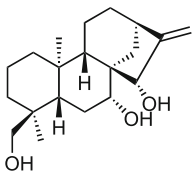
47: *ent*-kaur-16-en-19-oic acid



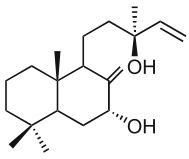
48: *ent*-kaur-16-en-7β-ol



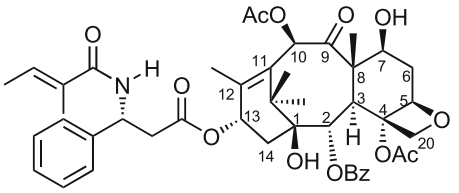
49: *ent*-7β,18-dihydroxykaur-16-ene



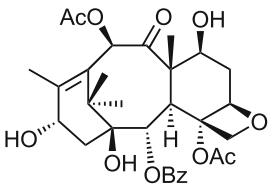
50: *ent*-7β,15β,18-trihydroxykaur-16-ene



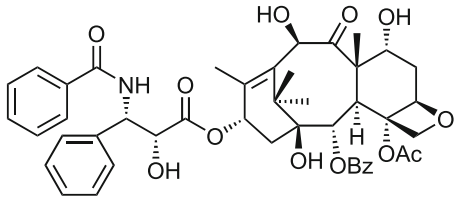
51: 7α-hydroxy-manool



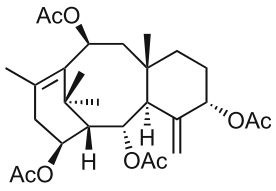
52: cephalomannine



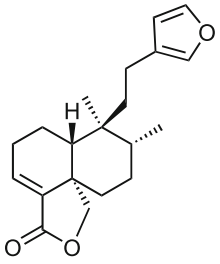
53: baccatin-III



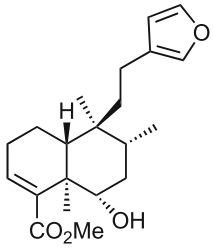
54:10-deacetyl-7-epi-taxol



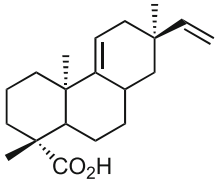
55: sinenxan



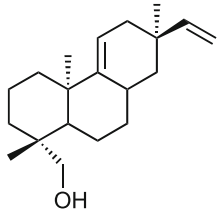
56: furanoclerodane dilactone



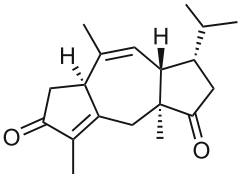
57: furanocleroda-19-oic acid methyl ester



58: *ent*-13-epi-pimara-9(11),15-dien-19-oic acid



59: *ent*-13-epi-pimara-9(11),15-dien-19-ol



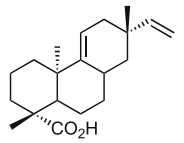
59d: hypoestenone

17.8 Structure Numbers and Names of Diterpenoids Including (Charts 6–13) and Table 1

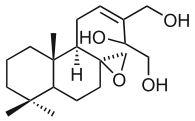
(**60**) (-)-Pimara-9(11),15-dien-19-oic acid
(**61**) 8β,17-Epoxy-14,15,16-trihydroxylabd-12*E*-ene (=aulacocarpin C)
(**62**) Dolabella-3,4-dien-10,18-diol
(**63**) Ajubractin A
(**64**) Ajugarin II
(**65**) Ajugarin IV
(**66**) Ajugarin III
(**67**) Ajugareptasone A
(**68**) 14ξ,15-Epoxylabda-8(17),12-dien-16-al
(**69**) Zerumin A
(**70**) Andrographolide A
(**71**) Coleon Q
(**72**) 4-epi-Triptoenzene
(**73**) Ovatodiolide
(**74**) *ent*-7α-Hydroxy-8(14),15-pimaradien-19-oic acid,
(**75**) *ent*-7-Oxo-8(14),15-pimaradien-19-oic acid
(**76**) *ent*-7β-Hydroxy-8(14),15-pimaradien-19-oic acid
(**77**) *ent*-2-Oxo-3-cleroden-15-oic acid
(**78**) Margacin
(**79**) Methylnimbiol
(**80**) Nimbiol
(**81**) Nimbosone
(**82**) Nimbonolone
(**83**) 8,11,13-Abietatrien-12,16-dihydroxy-3,7-dione (=margocinin)
(**84**) 8,11,13-Abietatrien-3β,12-dihydroxy-7-one (=margocilin)
(**85**) *ent*-3,13-Clerodadiene-15,16,17-triol
(**86**) 16-Hydroxybacchasalicyclic acid
(**87**) 16-Hydroxybaccasalicyclic acid-15-*O*-acetate
(**88**) 15,16-Dihydroxy-*ent*-3,13-clerodadiene-17-*O*-β-xylopyranoside
(**89**) *ent*-2α,3α-Dihydroxy-7-labden-15-oic acid
(**90**) Ballonigrine
(**91**) Bifurcadiol
(**92**) 7β,8β-Dihydroxypimar-15-en-3-one
(**93**) Caesalmine A
(**94**) Phanquinin A
(**95**) Macrophypene A
(**96**) 4*R*,9*S*,14*S*-4α-Acetoxy-9β,14α-dihydroxydolast-1(15),7-diene
(**97**) 12-Hydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial
(**98**) 1α,2α-Epoxyhinokione
(**99**) Camaecydin
(**100**) Cinncassiol A

(**101**) Cinncassiol-A-19-*O*-β-D-glucoside
(**102**) Cinnacassiol C
(**103**) Cinncassiol D_4
(**104**) Cinncassiol D_4-19-*O*-β-D-glucoside
(**105**) 8-Abieten-18-oic acid
(**106**) iso-Dehydropopulifolic acid
(**107**) epi-Hydroxy-populifolic acid
(**108**) β-Methoxypopulifolic acid
(**109**) Hydroxypopulifolic acid
(**110**) α-Methoxypopulifolic acid
(**111**) 2*R*,3*S*,4*R*,5*R*,6*S*,9*R*,10*R*,11*S*,13*S*,16*R*-6,19-Diacetoxy-3-[(2*R*)-2-acetoxy-2-methylbutyryloxy]-4,18:11,16:15,16-triepoxy-15-methoxy-7-clerodene-2-ol (=clerodendrin A)
(**112**) Mazambioside
(**113**) Mascarol
(**114**) Mascaroside
(**115**) Coleonol E
(**116**) Crotonolide A
(**117**) 18-Hydroxyaylthonic acid
(**118**) Dictyol A
(**119**) Dictyol H
(**120**) Epoxypachydictyol
(**121**) Diosbulbin N
(**122**) Dodonic acid
(**123**) 13,17-Epoxy-13-methyl-15-oxolabda-7-ene
(**124**) ent-15ξ-ethoxylabdan-3β,8α-dihydroxy-13(14)-en-15,16-olide
(**125**) Microthecaline A
(**126**) Euphoroylean A
(**127**) Phanginin A
(**128**) 3-Cleroden-15,18-diol
(**129**) 3-Clerodene-15,18-dioic acid (=haplociliatic acid)
(**130**) Coronarin E
(**131**) Gibberellin A_{67}
(**132**) Gibberellin A_{76}
(**133**) 19-Trachylobanoic acid
(**134**) Thujyl *ent*-trachyloban-19-oate
(**135**) Grandifloric acid
(**136**) α-Camphorene
(**137**) Metacamphorene
(**138**) *ent*-3,15-Dioxo-7β,14α-dihydroxykaur-16-ene
(**139**) Oridonin
(**140**) *ent*-Abieta-7-ene-3α,16,17,18-tetraol (=xerophilusin XIV)
(**141**) Curcusecone A
(**142**) Saviperone A

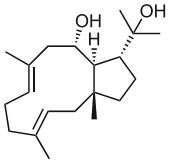
(**143**) 15,16-Dihydroxy-7,11-dioxopimar-8(9)-ene
(**144**) Nepetaefolin
(**145**) Preleosibirone A
(**146**) Euroabienol
(**147**) 12*S*-Hydroxymarrubiin
(**148**) Pretomentol
(**149**) Pretomexanthol
(**150**) Prezoapatanol
(**151**) Tomentanol
(**152**) Zoapatlin
(**153**) Obtusicrenone
(**154**) Momilactone A
(**155**) Momilactone B
(**156**) Momilactone C
(**157**) Ineketone
(**158**) 1(10),15-Rosandien-18-oic acid
(**159**) Spiroscutelone A
(**160**) 6-Hydroxy-11-deoxy-13-dehydrohetisane (=panicudine)
(**161**) *ent*-2α,6α,15β-Trihydroxykaur-15-ene
(**162**) Rabdokaurin C
(**163**) Rabdokaurin D
(**164**) Shikoccin
(**165**) Shikoccidin
(**166**) 8,12:15,16-Diepoxy-20-hydroxy-neo-cleroda-3,12(16),14-trien-18,19-olide (=hispanin A)
(**167**) Hispanin G
(**168**) Tanshinone II A
(**169**) Carnosic acid
(**170**) 6,7-*O*-Dimethyl-*epi*-rosmanol
(**171**) Safficinolide
(**172**) Sageone
(**173**) Scutebaicalin
(**174**) *ent*-1β-Hydroxy-7α-acetoxy-15β,16β-epoxykaurane
(**175**) Gibberellin A_{53}
(**176**) Teucrin A
(**177**) Teulolin A
(**178**) 16-Hydroxyferruginol
(**179**) 15*R*-12,16-Epoxy-8,11,13-abietatriene
(**180**) Totarol
(**181**) 7α-Hydroxytotarol
(**182**) 7β-Hydroxytotarol
(**183**) epi-Rosmanol
(**184**) *ent*-15α-Acetoxy-kaur-16-en-18-oic acid (=xylopic acid)
(**185**) 2-Oxo-kolavenic acid



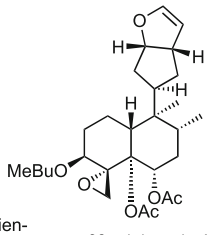
60: (-)-pimara-9(11), 15-dien-19-oic acid



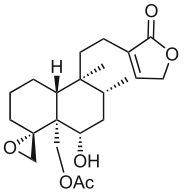
61: 8β,17-epoxy-14,15,16-trihydroxylabd-12*E*-ene (=aulacocarpin C)



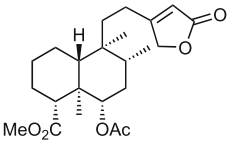
62: dolabella-3,7-dien-10,18-diol



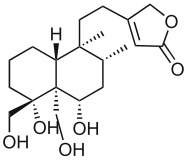
63: ajubractin A



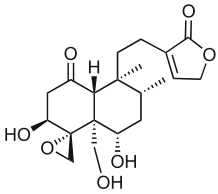
64: ajugarin II



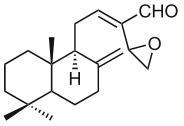
65: ajugarin IV



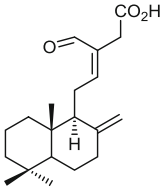
66: ajugarin III



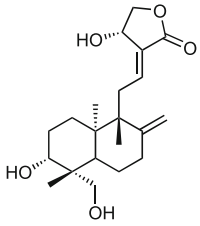
67: ajugareptasone A



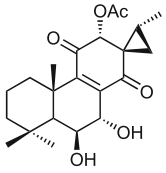
68: 14ξ15-epoxylabda-8(17),12-dien-16-al



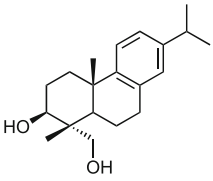
69: zerumin A



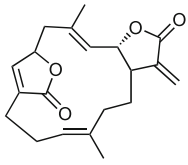
70: andrographolide A



71: coleon Q

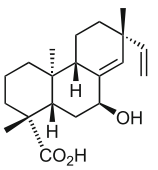


72: 4-*epi*-triptobenzene

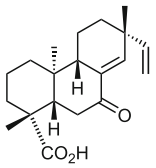


73: ovatodiolide

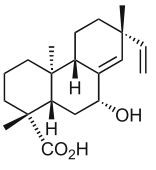
Chart 6 Structure numbers and names of diterpenoids including Table 1



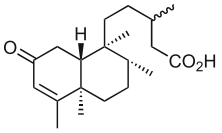
74: *ent*-7α-hydroxy-8(14),15-pimaradien-19-oic acid



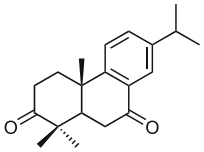
75: *ent*-7-oxo-8(14),15-pimaradien-19-oic acid



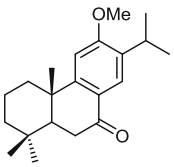
76: *ent*-7β-hydroxy-8(14),15-pimaradien-19-oic acid



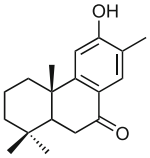
77: *ent*-2-oxo-3-cleroden-15-oic acid



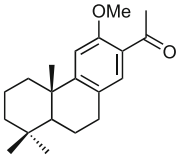
78: margocin



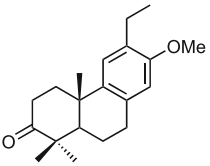
79: methylnimbiol



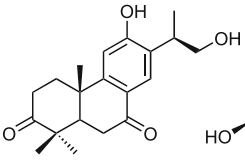
80: nimbiol



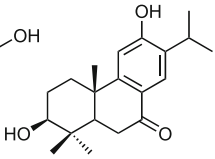
81: nimbosone



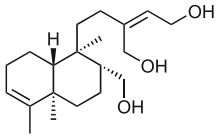
82: nimbonolone



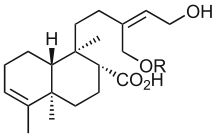
83: 8,11,13-abietatrien-12,16-dihydroxy-3,7-dione (=margocinin)



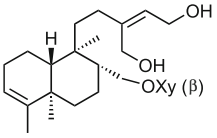
84: 8,11,13-abietatrien-3β,12-dihydroxy-7-one (=margocilin)



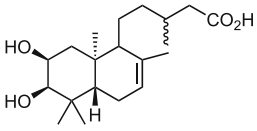
85: *ent*-3,13-clerodadiene-15,16,17-triol



86: R=H: 16-hydroxybacchasalicyclic acid
87: R=Ac: 16-hydroxybaccha salicyclic acid-15-*O*-acetate

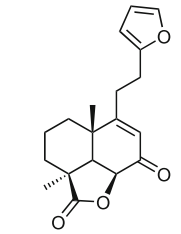


88:15,16-dihydroxy-*ent*-3,13-clerodadiene-17-*O*-β-xylopyranoside

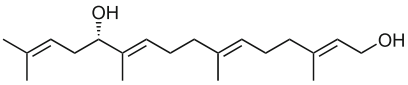


89: *ent*-2α,3α-dihydroxy-7-labden-15-oic acid

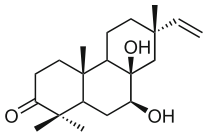
Chart 7 Structure numbers and names of diterpenoids including Table 1



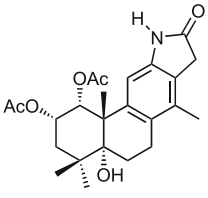
90: ballonigrine



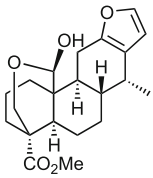
91: bifurcadiol



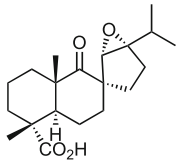
92: 7β,8β-dihydroxypimar-15-en-3-one



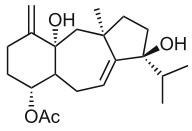
93: caesalmine A



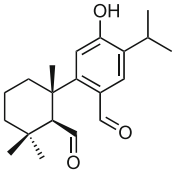
94: phanginin A



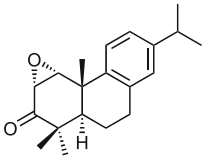
95: macrophypene A



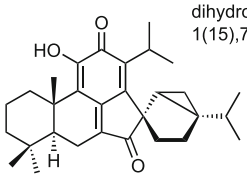
96: 4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene



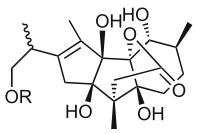
97:12-hydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial



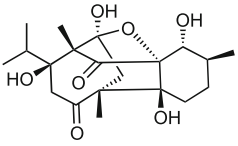
98:1α,2α-epoxyhinokione



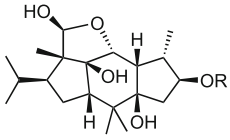
99: camaecydin



100: R=H: cinncassiol A
101: R=Glc (β-): cinncassiol-A-19-*O*-β-*D*-glucoside

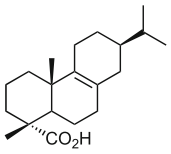


102: cinnacassiol C

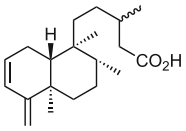


103: R=H: cinncassiol D_4
104: R=Glc (β-): cinncassiol D_4-19-*O*-β-*D*-glucoside

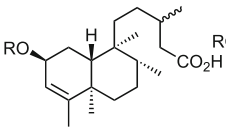
Chart 8 Structure numbers and names of diterpenoids including Table 1



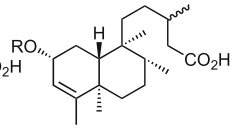
105: 8-abieten-18-oic acid



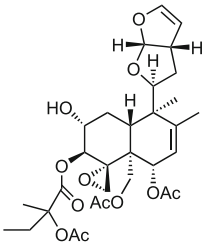
106: iso-dehydro-populifolic acid



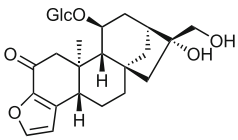
107: R=H: *epi*-hydroxy-populifolic acid
108: R=Me: β-methoxy-populifolic acid



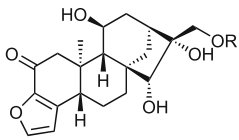
109: R=H: hydroxypoplifolic acid
110: R=Me: α-methoxy-populifolic acid



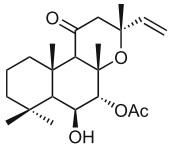
111: 6,19-diacetoxy-3-[(2*R*)-2-acetoxy-2-methylbutyryloxy]-4,18:11,16:15,16-triepoxy-15-methoxy-7-clerodene-2-ol (=clerodendrin A)



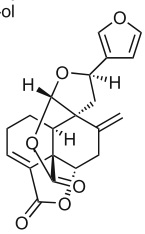
112: mazambioside



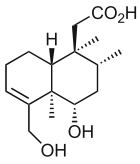
113: R=H: mascarol
114: R=Glc (β-): mascaroside



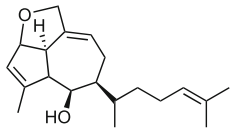
115: coleonol E



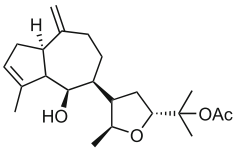
116: crotonolide A



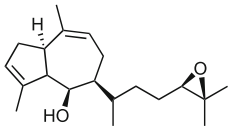
117: 18-Hydroxy-aylthnoic acid



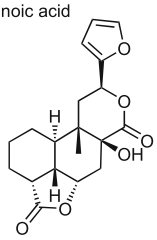
118: dictyol A



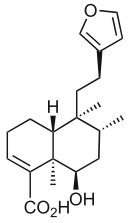
119: dictyol H



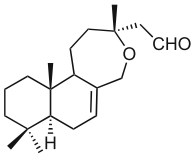
120: epoxypachdictyol A



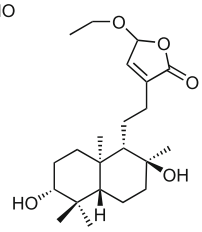
121: diosbulbin N



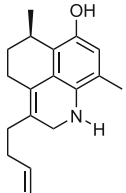
122: dodonic acid



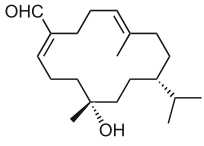
123: 13,17-epoxy-13-methyl-15-oxolabda-7-ene



124: *ent*-15ξ-ethoxylabdan-3β, 8α-dihydroxy-13(14)-en-15,16-olide

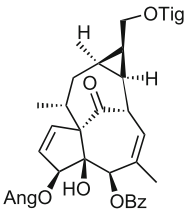


125: Microthecaline A

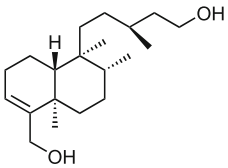


126: euphoroylean A

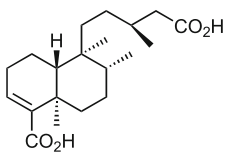
Chart 9 Structure numbers and Names of diterpenoids including Table 1



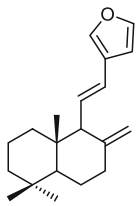
127: phanginin A



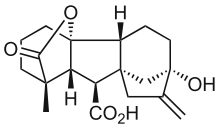
128: 3-cleroden-15,18-diol



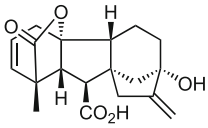
129: 3-clerodene-15,18-dioic acid (=haplociliatic acid)



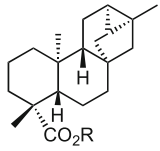
130: coronarin E



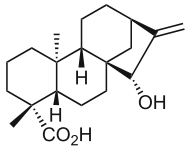
131: gibberellin A_{67}



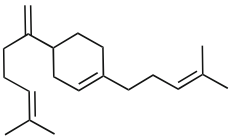
132: gibberellin A_{76}



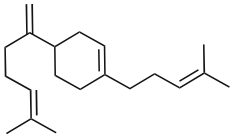
133:19-trachilobanoic acid
134: thujyl ent-trachyloban-19-oate



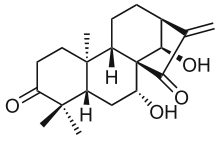
135: grandifolic acid



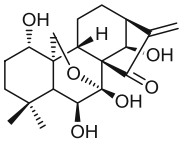
136: α-Camphorene:



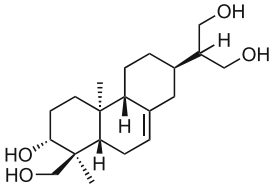
137: metacamphorene



138: *ent*-3,15-dioxo-7β,14α-dihydroxykaur-16-ene

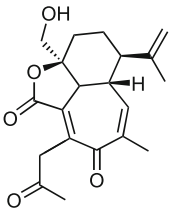


139: oridonin

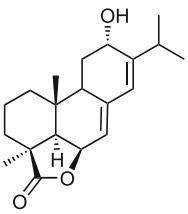


140: *ent*-abieta-7-ene-3β,16,17,18-tetraol (= xerophilusin XIV)

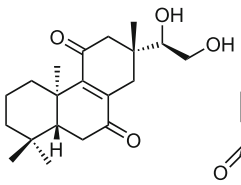
Chart 10 Structure numbers and names of diterpenoids including Table 1



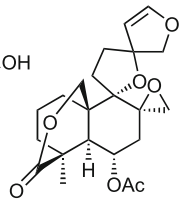
141: curcusecone A



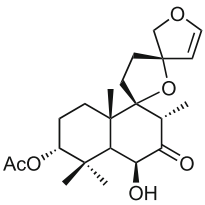
142: saviperone A



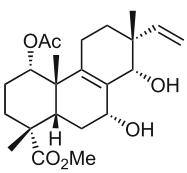
143: 15,16-dihydroxy-7,11-dioxopimar-8(9)-ene



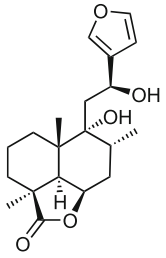
144: nepetaefolin



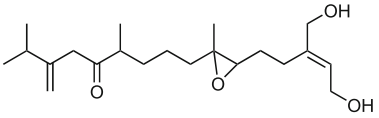
145: preleosibirone A



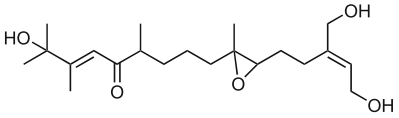
146: euroabienol



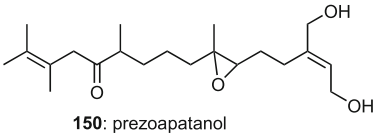
147: 12*S*-hydroxymarrubiin



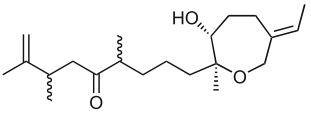
148: Pretomentol



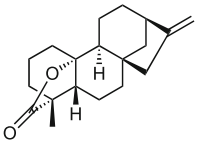
149: pretomexanthol



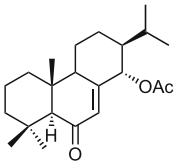
150: prezoapatanol



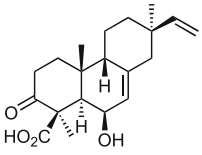
151: tomentanol



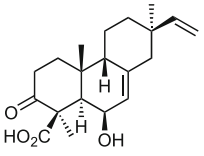
152: zoapatlin



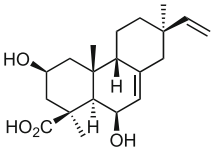
153: obtusicrenone



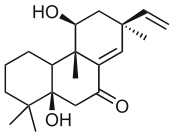
154: momilactone A



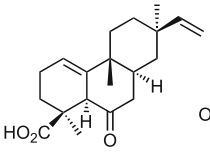
155: momilactone B



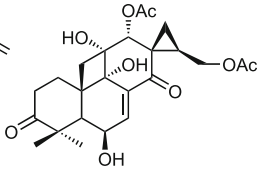
156: momilactone C



157: ineketone

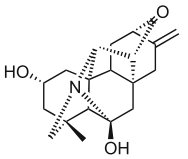


158: 1(10),15-rosadien-18-oic acid

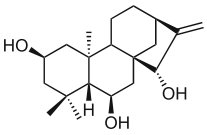


159: spiroscutelone A

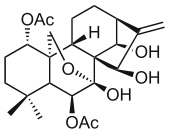
Chart 11 Structure numbers and names of diterpenoids including Table 1



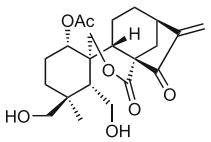
160: 6-hydroxy-11-deoxy-13-dehydrohetisane (= panicudine)



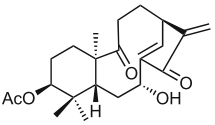
161: *ent*-2α,6α,15β-trihydroxykaur-16-ene



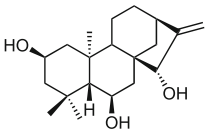
162: rabdokaurin C



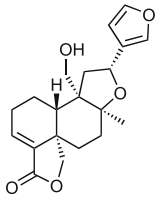
163: rabdokaurin D



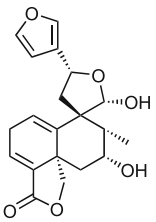
164: shikoccin



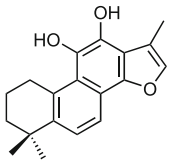
165: shikoccidin



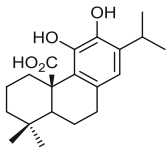
166: 8,12:15,16-diepoxy-20-hydroxy-neocleroda-3,12(16),14-trien-18,19-olide (=hispanin A)



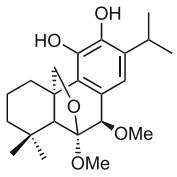
167: hispanin G



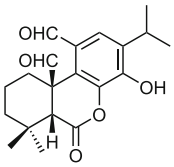
168: Tanshinone II A



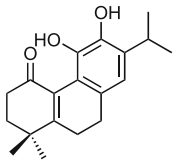
169: carnosic acid



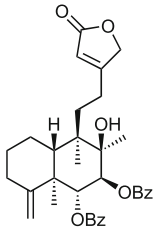
170: 6,7-*O*-dimethyl-*epi*-rosmanol



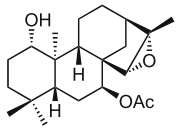
171: safficinolide



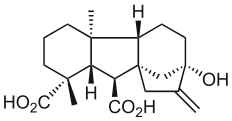
172: sageone



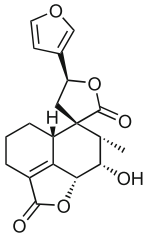
173: scutebaicalin



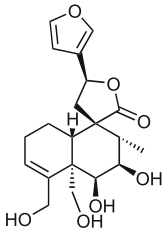
174: *ent*-1β-hydroxy-7α-acetoxy-15β,16β-epoxykaurane



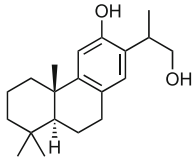
175: gibberellin A_{53}



176: teucrin A

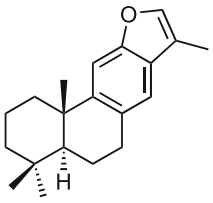


177: teulolin A

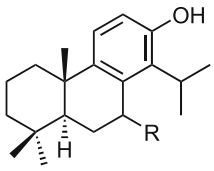


178: 16-Hydroxyferruginol

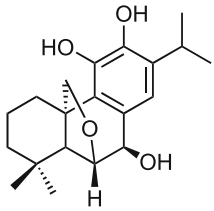
Chart 12 Structure numbers and names of diterpenoids including Table 1



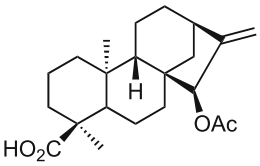
179: 15*R*,12,16-epoxy-8,11,13-abietatriene



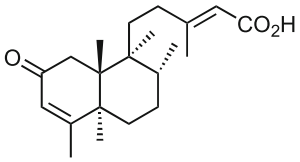
180: R=H: totarol
181: R=OH (α-): 7α-hydroxytotarol
182: R=OH (β-): 7β-hydroxytotarol



183: *epi*-rosmanol



184: ent-15α-acetoxy-kaur-16-en-18-oic acid (=xylopic acid)



185: 2-oxokolavenic acid

Chart 13 Structure numbers and names of diterpenoids including Table 1

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Tea Catechins

18

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Abstract

Tea, a water infusion of leaves from *Camellia sinensis* (L.) Kuntze, is the oldest, most consumed beverage worldwide. Archeologists have recently discovered traces of tea drinking dating to around 206 B.C. There are five major types of tea, differing in production processes and, therefore, in chemical composition: green tea, white and yellow teas, oolong tea, black tea, and dark tea. Compounds identified in green tea are involved in several biological activities and can have various health-promoting functions, which can include antioxidant, anti-inflammatory, antiviral, anticancer, antidiabetic, and anti-obesity activities, as well as activities against CVD and neurodegenerative diseases, and many others. These beneficial effects are mainly ascribed to tea catechins, bioactive constituents belonging to the flavonoid family (flavan-3-ols subclass), which represent approximately 70% of all polyphenols in tea. However, the maximal concentrations of the catechins detected in blood in human subjects or animals after oral ingestion are in submicromolar or low micromolar levels, which indicate that only small amounts are absorbed and passed into the blood. Chapter "Tea Catechins" describes the chemical composition of *C. sinensis* leaves and the beneficial effects on human health upon tea consumption, focusing on tea catechin bioactive constituents. This chapter also takes into account their bioavailability, toxicological aspects, and safety profiles. Furthermore, recent applications of tea catechins in the food market are presented.

Keywords

Camellia sinensis · Tea catechins · Phytochemistry · Bioactive compounds · Health properties · Bioavailability

18.1 Introduction

Tea is the most consumed beverage worldwide, and tea plant from which it is derived, *Camellia sinensis* (L.) Kuntze, is currently grown in over 50 countries of the world. The main producers of tea are China and India, with 2,473,443 t and 1,325,050 t harvested, respectively, in 2017 alone, followed by Indonesia, Iran, Turkey, Vietnam, Kenya, and Sri Lanka (FAOSTAT 2019).

18.1.1 Botanical Description

According to its botanical classification, *C. sinensis* belongs to the *Theaceae* family which are woody plants with simple leaves, which may be more or less leathery and have smooth or toothed edges, widespread in temperate regions. *C. sinensis* is a small, highly branched tree, standing at 5–10 m tall, characterized by spaded oval evergreen leaves with toothed edges, which are soft and velvety when young and coriaceous when they are ripe. The shape and size of the leaves are distinctive characteristics in establishing the variety of the plant. The flowers have six to nine petals, are creamy white, and are solitary or grouped in twos or threes, while the fruit consists of a small woody capsule with a few dark, flattened seeds. The oil pressed from these seeds is used in cooking, due to its sweet taste. The plants are cultivated to the height of 1 m to facilitate harvesting, which occurs almost exclusively by hand in order to obtain the finest products and provides for the collection of unopened terminal buds, called pekoe, and the first leaves, young and soft, which produce one of the highest quality tea infusions. Shortly preceding the first harvest, which takes place between April and May, it is traditional practice to obscure the plant to encourage an increase in the content of flavonoids, sugars, and other molecules, which are representative of the quality of the tea, creating the necessary conditions for obtaining a sweeter and more precious product. The products of the first harvest differ from later harvests as they may include both the first leaves of the new season and the remaining leaves of the previous season.

The varieties of tea plant with commercial value are *C. sinensis* var. *sinensis* (called China type) and *C. sinensis* var. *assamica* (called Assam type, deriving from the Northeast state of India). Recent publications on the genetic profiling of tea have clarified that other tea varieties, e.g., *C. assamica* subsp. lasiocalyx Planch (called Cambod type), which have been considered to be synonyms of China and Assam tea types, are actually hybrids. Indeed, as breeding techniques have evolved, artificial pollination and hybridization are also steadily growing in importance.

18.1.2 History

A Chinese legend states that tea was discovered accidentally by Emperor Shen Nong in 2700 B.C., when camping under a tree after a long trip, some tea leaves fell in his pot of boiling water. However, tea as a beverage was first mentioned in Erh Ya,

a Chinese book dating from 350 B.C. Archeologists have recently discovered evidence of tea drinking in the excavation of "Han Yang Ling Mausoleum" in the Shaanxi province of China, revealing the diffusion of tea in the Han dynasty (206 B.C.). Tea drinking spread to Japan around the sixth century, as a privilege of high society, and became more widely popular about 700 years ago. The occidental consumption of tea arose from the tea trade between China and Holland, from which the English first gained an appreciation for drinking tea. In the seventeenth century, England was the first player in tea commerce imported from India, imposing restrictive trade conditions and strict taxation on the colonies. That is why, following the approval of the Tea Act in 1773, American colonials threw the tea cargo of British ships overboard off the coast of Boston (Boston Tea Party). Globally, tea consumption differs in the types of tea consumed and in the manner of consumption. Traditionally, green tea or oolong tea is preferred in Asia, whereas black tea is the customary beverage in much of the rest of the world, especially Europe, North America, and North Africa. Black tea is usually taken hot with milk in the Anglo-Saxon regions, whereas Americans prefer ice tea, introduced in 1904 at the St. Louis World's Fair, as a result of a particularly hot season.

18.1.3 Traditional Medical Application

Although the diffusion of tea drinking primarily occurred due to its sensorial properties, tea was initially consumed for its health properties, as with other herbal remedies in traditional Chinese medicine. In fact, it was mostly consumed for its digestive and detoxifying properties. Nevertheless, scientific investigation on this topic only began in the last decade of the eighteenth century. Even in India, tea was used traditionally as a medicinal plant before British colonization, after which tea was used as a drink for pleasure.

18.1.4 Tea Classification and Production Processes

Although the properties of tea differ between plant cultivars, climatic conditions, and producing regions, the post-harvest conditions have the highest impact on the variability of its chemical profiles and, consequently sensorial properties. Tea beverages are divided into five types: green tea, white and yellow teas, oolong tea, black tea, and dark tea (or pu-erh tea). Contrary to popular belief, every type is made from *C. sinensis* leaves. In fact, this classification is only based on the different production processes: non-fermented (green tea), lightly fermented (white and yellow teas), semi-fermented (oolong tea), fully fermented (black tea), and post-fermented (dark tea) (Fig. 1) (Tenore et al. 2015). Generally speaking, it's possible to subdivide the production process into a number of principal steps as reported below.

Picking: the operation in which the operator chooses buds or young or mature leaves.

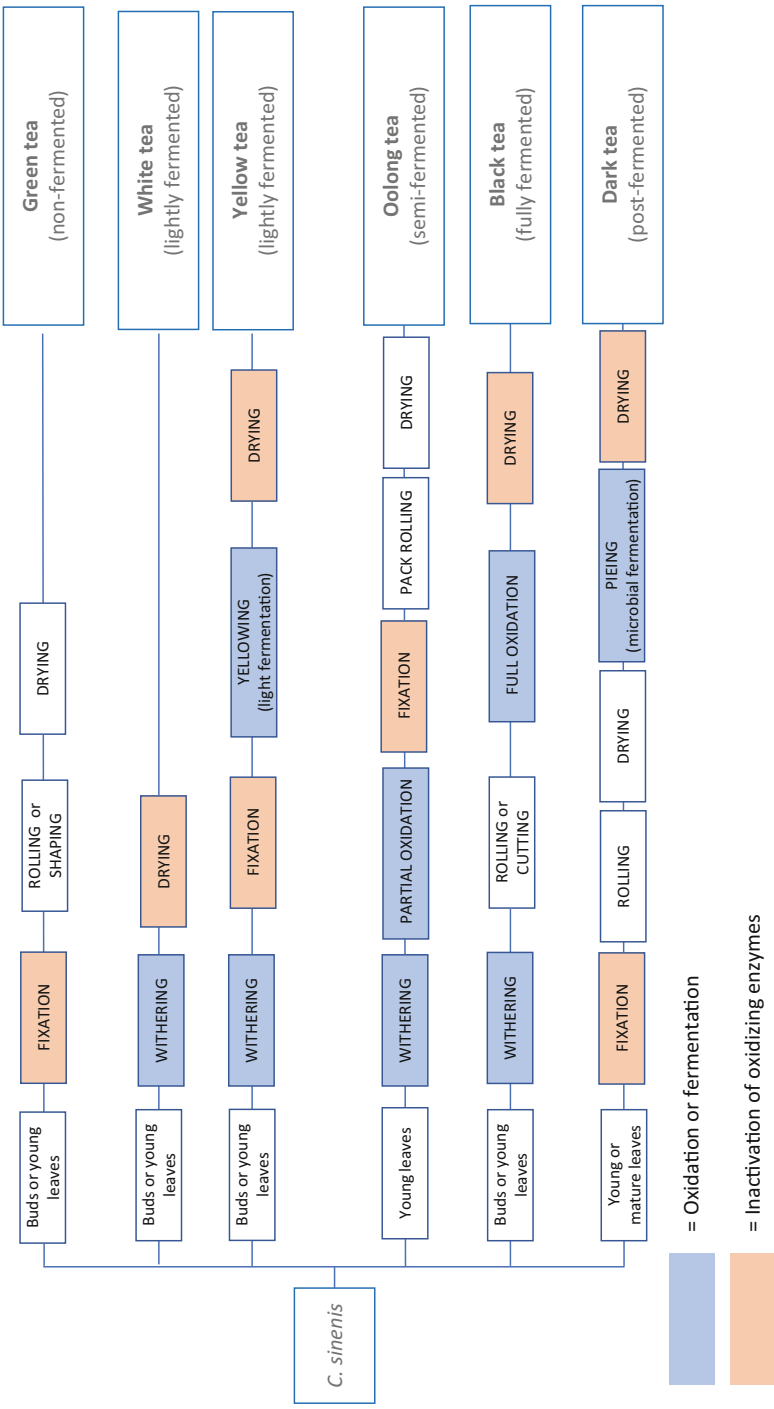


Fig. 1 Tea production processes

Withering: in this operation the tea leaves are spread in a series of enclosed or open troughs fitted with perforated trays under forced air circulation. During this period, the leaves lose some of their moisture, from approximately 70–80% to 60–70% of fresh weight and become more flexible for rolling. The withering operation has a strong impact on the chemical profile of the end product, as it catalyzes oxidative reactions resulting in slight oxidation of the leaves.

Fixation: is a step in the production of green tea, leading to the inactivation of enzymes and cessation of oxidation, with a significant impact on color and taste. To do this, leaves are slightly warmed to approximately 85 °C for short time (min) under dry or wet conditions.

Rolling: the shaping of tea leaves by hand or rolling machines. This operation leads to the release of essential oils from cell walls.

Pieing: this step involves the microbial fermentation of the leaves, induced by incubation at about 30 °C under moist conditions.

Crushing or cutting: the process that allows a greater intensity of oxidative reactions by increasing the exposed surface.

18.2 Bioactive Constituents

The chemical composition of tea leaves varies broadly on the basis of plant cultivar, growing environment, harvest season, and manufacturing conditions. The main bioactive constituents of tea leaves are polyphenols and methylxanthines, including catechins (25–35% D.W.), caffeine (3.5% D.W.), theobromine (0.15–0.2% D.W.), and theophylline (0.02–0.04% D.W.). Furthermore, organic acids (1.5% D.W.), chlorophyll (0.5% D.W.), theanine (4% D.W.), and free amino acids (1–5.5% D.W.) are present in tea leaves, in addition to a variety of other bioactive components in small amounts, such as flavones, flavonols (mainly quercetin, kaempferol, myricetin), phenolic acids, carbohydrates, minerals, vitamins, and enzymes.

It has been long established that the oil obtained from tea seeds is rich in antioxidant compounds. Recent studies have identified a significant amount of catechins in tea seed oil, suggesting that this product could be a promising source of bioactive compounds, such as catechins. In particular, George and colleagues showed that the total catechin content is about 4.8 ± 0.9 to $9.8 \pm 0.25 \times 10^{-3}$ (expressed as % of flavonoids) in seed oil from various tea clones (George et al. 2015).

18.2.1 Tea Catechins

Taking into account evidence presented in scientific literature, beneficial effects of tea are mainly attributable to tea catechins, which represent approximately 70% of all polyphenols in tea. Tea catechins, which are abundant in the young leaves and buds of tea plants, belong to the flavonoid family and to the subclass of flavan-3-ols. The main tea catechins are (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-

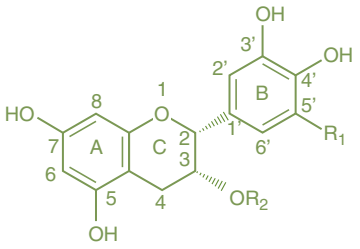
epigallocatechin-3-gallate (EGCG). Furthermore, tea contains (+)-catechin (C), (+)-gallocatechin (GC), (−)-gallocatechin-3-gallate (GCG), and (+)-catechin-3-gallate (CG).

These eight molecules are derived from the absence or presence of a gallyl substituent on the following four catechins: (+)-catechin, (−)-epicatechin, (+)-gallocatechin, and (−)-epigallocatechin.

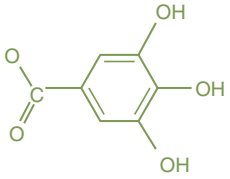
18.2.1.1 Chemical Structure of Tea Catechins

The structural basis of catechin molecules consists of two benzene rings, the A- and B-rings, which are similar to resorcinol and catechol moieties, respectively. Furthermore, catechins contain a dihydropyran heterocycle with a hydroxyl group on carbon 3, the C-ring. Catechins show two chiral centers on the C-ring against carbons 2 and 3. Hence, it has four diastereoisomers, of which two stereoisomers are in a *trans* configuration (referred to as catechin) and the other two in a *cis* configuration (referred to as epicatechin), as shown in Fig. 2.

The results obtained from in silico and in vitro studies on structure-activity relationships reveal that the molecular structure of catechins plays an important role in its antioxidant properties. As stated in these investigations, the antioxidant activity of tea catechins is mainly determined by the number and position of hydroxyl groups. Moreover, an increase in antioxidant activity could be linked to the presence of several structural elements, such as an o-dihydroxyl catechol structure in the B-ring, and the presence of unsaturation and a 4-oxo group in the



Main catechin structure



Gallate group

	R1	R2
EC	H	H
EGC	OH	H
ECG	H	Gallate group
EGCG	OH	Gallate group

Fig. 2 Catechins molecular structure

C-ring. In addition, the antioxidant activity of catechins could be facilitated by the 2,3-double bond in the C-ring, together with 4-oxo function in the C-ring, since these enable electron delocalization from the B-ring, or by the hydroxyl groups at positions 3 and 5, since these provide the hydrogen bonding to the 4-oxo group in the C-ring.

18.2.1.2 Biosynthesis of Tea Catechins

Catechins are synthetized through the flavonoid pathway, starting from 4-coumaroyl CoA, which, in turn, is derived through the phenylpropanoid pathway. The first enzyme involved in the flavonoid pathway is *chalcone synthase* (CHS) which catalyzes the condensation of 4-coumaroyl CoA and three units of malonyl-CoA, to form chalcone, also called naringenin chalcone. Subsequently, *chalcone isomerase* (CHI) catalyzes the isomeration of chalcone to naringenin, the corresponding flavanone. From this point the flavonoid pathway branches in different ways, which result in the synthesis of different classes of flavonoids: flavones, flavonols, flavan-3-ols, and anthocyanins.

Where flavan-3-ols are concerned, non-esterified catechins (C, EC, GC, and EGC) are produced in steps involving sequential reactions catalyzed by leucoanthocyanidin 4-reductase (LAR), anthocyanidin synthase (ANS), and anthocyanidin reductase (ANR), while esterified catechins (EGCG and ECG) are produced from EGC and EC by flavan-3-ol gallate synthase (FGS). The detailed flavonoid pathway is reported in Fig. 3.

18.2.2 Factors Determining a Modification of Tea Catechin Profile

There are various factors which may modify the profile of tea catechins, through epimerization, hydrolysis, oxidation or condensation, and oligomerization reactions. These factors, which are reported in further detail below, can occur during the growth of the plant, during post-harvest operations or even during infusion.

18.2.2.1 Growing Conditions: Impact on Tea Catechin Profile

The impact of growing conditions on the chemical profile of tea leaves is significant. Therefore, this section briefly covers the effects of shade techniques and the influence of an ethylene modified atmosphere on tea plants.

Shade

The production of shade-grown green teas was developed in Japan, following the spreading of tea from China at the hands of Buddhist monks in the sixteenth century. Tea bushes are progressively kept under rice-straw screens in April, the time corresponding with the opening of the first leaves. After 20 days, tea leaves are harvested by hand, immediately steamed to inactivate oxidative enzymes, and then dried in a specific tunnel. At this point, if the dried leaves are pulverized, matcha tea is produced, while in cases where the leaves are kept whole, the final products are generally named *shade teas* (e.g., gyokuro tea). Matcha, known as the "ceremony

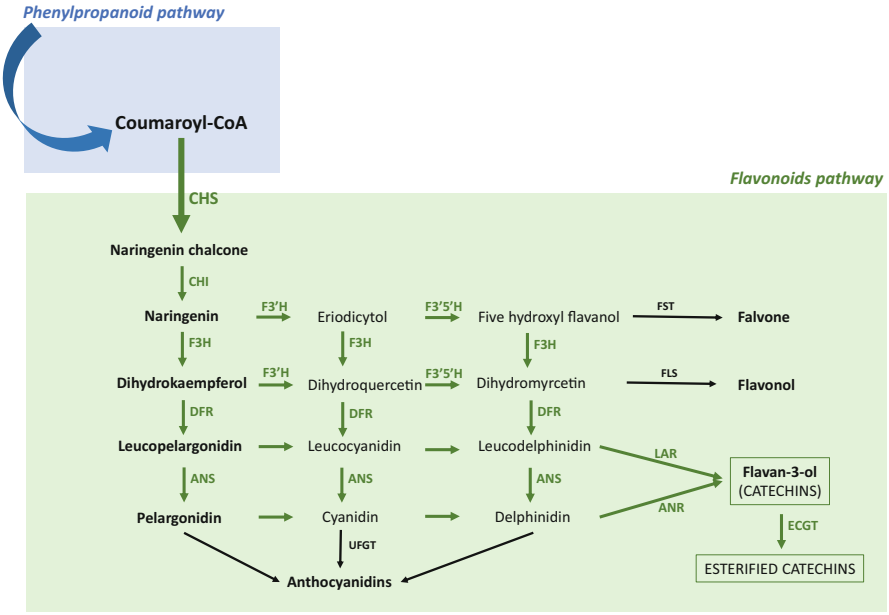


Fig. 3 Biosynthesis of catechins. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3′-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′, 5′-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; UFGT, UDP-glucose flavonoid 3-O-glucosyltransferase; FST, flavonol 4′-sulfotransferase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; ECGT, epicatechin glucosyltransferase

tea," and the other *shade teas* are characterized by a brilliant light green color, which actually derives from the shade process. Tea plants, as with other species, are able to enhance their photosynthetic apparatus when grown under shade conditions. Indeed, young leaves grown in shade conditions accumulate a greater amount of chlorophyll than in other teas (called *light teas*). Another interesting characteristic of *shade teas* is their high content of amino acids, such as theanine, which gives them a unique sweet taste. On the other hand, it is well-known that the solar radiation regulates the biosynthesis of phenylpropanoids. So, the flavanol content of *shade teas* is lower than that of *light teas*, from about 10% to 13.1% of flavonoids, respectively. The amount of catechins of matcha (*shade tea*) compared to green tea (*light tea*) is reported in Table 1, which shows data from a research article carried out by Weiss and Anderton (Weiss and Anderton 2003). While Japan remains the leading consumer of matcha tea, followed by China, consumption of matcha in the United States and Europe is expected to grow due to its recent use in different kind of smoothies, hot milk beverages, and alcoholic beverages such as matcha beer. It also important to note that while normally tea leaves are infused with water and the extract is consumed, in the case of matcha tea, powdered tea leaves are added to water and the leaves are thus consumed as part of the beverage. For

Table 1 Amount of catechins in matcha (shade tea) compared to green tea (light tea) (Weiss and Anderton 2003)

Compound	Green tea (mg/g)	Matcha tea (mg/g)
Caffeine	3.9 ± 0.35	23.9 ± 0.34
Catechin	6.2 ± 0.01	Under limit of detection
EGC	6.2 ± 0.05	0.75 ± 0.04
EGCG	0.42 ± 0.05	0.32 ± 0.03
EC	4.4 ± 0.30	2.4 ± 0.11
ECG	0.10 ± 0.02	0.25 ± 0.03

this reason, the intake of bioactive constituents is higher for matcha than in other types of tea, assuming the same drug-solvent ratio.

Ethylene

Ethylene, a plant hormone that affects the growth and development of plants, plays a crucial role in plant signal transduction. In some plant species, ethylene signalling is involved in plant defenses by inducing the accumulation of secondary metabolites and enhancing polyphenol oxidase activity. A recent research article aiming to elucidate the effects of ethylene on the synthesis of phenolic compounds in tea seedlings showed that this plant hormone significantly enhanced the total content of catechin. In more detail, tea seedlings were exposed to a modified atmosphere, which presented a large amount of 1-aminocyclopropane-1-carboxylic acid (ACC), a direct precursor of ethylene. The results of chromatographic analyses indicate that this ACC treatment increases the galloylation of EGC and EC. Furthermore, ACC exposure enhances anthocyanin synthesis. However, the antioxidant enzyme activity assay showed that the activity of catalase (CAT), superoxide dismutase (SOD), and total peroxidase (POX) decreases, while ascorbate peroxidase (APX) activity is increased.

These findings elucidate that ethylene signalling is involved in the modulation of the accumulation of secondary metabolites and could thus be useful in increasing the healthy properties of tea products.

Post-Harvested Conditions: Impact on Tea Catechin Profile In general, it can be stated that post-harvest conditions have the largest influence on tea catechin profile, since the various processes yielding the various types of teas belong to these kinds of operations.

Anaerobic Conditions

In 1987, a Japanese scientist, named Dr. Tsushida Tojiro, accidentally discovered that freshly picked tea leaves exposed to anaerobic conditions in a nitrogen-rich modified atmosphere accumulated GABA, synthetized from glutamic acid. Thanks to Dr. Tojiro's discovery, a new type of tea, GABA tea, was created. According to the standards defined by the Japanese authorities, GABA tea must have a GABA amount of at least 150 mg/100 g of dry tea. Today, the GABA tea

Table 2 Content of polyphenolic compounds in GABA and green teas (Wang et al. 2006)

Compound	Green tea (mg/g)	GABA tea (mg/g)
Caffeine	3.22 ± 1.14	3.34 ± 1.36
Catechin	0.76 ± 0.31	0.73 ± 0.46
EGC	2.85 ± 1.67	3.71 ± 2.06
EGCG	4.69 ± 1.55	3.26 ± 1.78
EC	0.62 ± 0.23	0.44 ± 0.67
ECG	0.85 ± 0.64	0.66 ± 0.58
GC	2.48 ± 1.24	3.17 ± 2.01

production process also involves the shade operation of tea, derived from the observation that shade growing tea plants have increased glutamic acid content. GABA tea is appreciated for its unique sensorial properties and especially for the great deal of evidence for its health properties, such as sleep-promoting effects, antihypertensive activity, and antidepressive-like effects (Di Lorenzo et al. 2016; Daglia et al. 2017). A recent study explains the mechanisms by which anoxia increases GABA concentration by about 20 times that of untreated tea. The results suggest that the increase in GABA is due to the increase in activity of the enzymes glutamate decarboxylase and diamine oxidase, and the increase of putrescine and spermine, two substrates for GABA production (Liao et al. 2017). In addition to its influence on GABA content, the exposure of picked tea leaves to anaerobic conditions leads to a variation in the catechin profile of GABA tea. Wang et al. reported that, compared to green tea, GABA tea shows significantly lower levels of EGCG and EC, while GC, C, EGC, and ECG do not differ (Table 2) (Wang et al. 2006).

Decaffeination

As in the case of coffee, various processes exist to yield a decaffeinated tea. Nowadays, ethyl acetate or methylene chloride assisted solvent extraction and supercritical fluid extraction are the most used methods for producing decaffeinated tea, as these avoid toxic organic solvents, such as chloroform, ethyl hexanoate, and propyl acetate or organochlorine solvent. Other processes include the separation of caffeine by the use of resin and charcoal. An innovative approach for the degradation of caffeine is by microbial treatment with *Pseudomonas* and *Aspergillus* spp. However, it is important to take into account that these environment-friendly processes are characterized by a high cost. Furthermore, some methods, such as decaffeination processes using hot water or cosolvent-assisted supercritical fluid extraction, lead to the loss of tea catechins. Park and colleagues first elucidated this phenomenon, observing that during the extraction of caffeine using the supercritical CO_2 extraction method, the loss of catechins was high in decaffeinated tea, while the loss was low in semi-decaffeinated tea, with low degree of decaffeination (Park et al. 2007). Therefore, the decaffeination process represents a challenge to the crucial retention of catechins.

Withering

As explained in Sect. 1.4, withering is a process aimed at reducing the moisture in tea leaves, which leads to an increase in the concentration of cell enzymes, resulting in activation of catechol oxidase and phenol oxidase. These enzymes trigger the oxidative reaction responsible for decreasing the amount of catechins, and increasing the concentrations of theaflavins and thearubigins. This is why white, oolong, and black teas, which are subjected to withering processes, are characterized by low catechin levels. Furthermore, withering results in the enhancement of caffeine, amino acids, and sugar content, while the concentration of chlorophyll decreases.

Fermentation

As with withering, the fermentation of tea leaves involves the oxidation process, mostly induced by the activity of endogenous and exogenous enzymes, which in this case also cause the formation of polymeric theaflavins and thearubigins. Oolong tea is a partially fermented tea, which requires the inactivation of enzymes after about an hour of fermentation. Oolong tea contains a mixture of monomeric polyphenols and higher molecular weight theaflavins. Black tea is produced by the extensive oxidation of polyphenols, with the inactivation of enzymes being induced following 3–6 h of fermentation, resulting in the accumulation of a great amount of thearubigins and theaflavins. As green tea is non-fermented, this contains a relatively unmodified quantity of monomeric catechins, with the polyphenols-oxidase enzymes being immediately inactivated after picking by means of dry heat or steam as part of the fixation process.

18.2.2.2 Brewing Methods: Impact on Tea Catechin Profile

Even the infusion methods for tea have a significant impact on the catechin profile, mainly due to differences in stability of pH, temperature, and storage time.

Tea catechins solubilized in water are stable at 37 °C for 7 h, while at 98 °C there is a 20% decrease after the same time. When EGCG is submitted to the temperature of 120 °C for 20 min, the epimerization of EGCG to (−)-gallocatechin gallate (GCG) is observed. EGCG, ECG, EGC, and EC are most stable at pH 4–5 with decreasing stability at lower and higher pH. At higher pH, the epimerization into GCG and CG increases. Thus, the relatively high amount of GCG found in some drinks is most likely due to epimerization of EGCG. A progressive decrease in total levels of catechins was reported during storage of green tea bags at 20 °C for 6 months. This phenomenon is due in part to losses in the most abundant catechins. Indeed, EGCG decreased by 28% and ECG by 51%, probably due to auto-oxidation reactions which increase with pH. So, it is essential to take into account the degradation of tea catechins during production, storage, and transport.

In addition, the number of infusions influences the catechin profile of brewed tea. In a study conducted by Yang and colleagues, the highest catechin extraction yield was obtained from the first or second infusion, depending on the applied temperature (Yang et al. 2007).

Another factor is the water quality, the subject of a recent research article considering the extraction yield of EGCG and EGC. These results indicated that

the choices of reverse osmosis water, packaged drinking water, and ultrapure water are the best, instead of tap water and soft water.

A particularly remarkable phenomenon takes place when tea beverages cool down, known as *creaming*, that consists of the precipitation of an insoluble catechin-caffeine complex, resulting in a decrease in the total catechin content of the beverage. A study from Ishizu and colleagues revealed that the esterified forms of catechins (EGCG and ECG) are more subject to creaming (Ishizu et al. 2016). Colon and Nerin observed that catechins and caffeine are re-solubilized at low pH values (Colon and Nerin 2014).

Moreover, Xu et al. found that the addition of sugars (30 g/mL), such as sucrose and fructose, decreases the creaming phenomenon (Xu et al. 2017).

18.2.3 Catechins Distribution in Tea Products

The content of catechins in a given tea beverage is strictly related to its subtype (green, white, oolong, and black) due to the strong impact of the fermentation process on catechin molecules. Green tea, which is produced without fermentation, has the highest amount of catechins: approximately 130 mg/100 g of green tea brewed (1 g dried tea leaves per 100 mL boiling water), subdivided as reported in Tables 1 and 2, of which the most representative catechin is EGCG (70.2 ± 4.08 mg/100 g) (USDA 2007). The catechin content of the other tea subtypes decreases with the degree of fermentation. So, considering EGCG as the most abundant catechin, an analysis of literature and market data conducted by the US Department of Agriculture (USDA) showed that white tea infusion contains an average of 42.45 ± 15.47 mg/100 g of EGCG, oolong tea infusion contains an average of 34.48 ± 4.76 mg/100 g of EGCG, and black tea infusion contains an average of 9.36 ± 0.46 mg/100 g of EGCG (USDA 2007). As discussed above, the fermentation process increases the aerobic oxidation catalyzed by enzymes, which is then followed by a succession of chemical condensations.

18.3 Bioavailability and Metabolism

Metabolism is the main limiting step in the beneficial activities of phenolic compounds on human health. The bioavailability and metabolism of tea molecules, especially tea catechins, has been studied since the early 2000s. However, human data are still lacking at present.

18.3.1 Absorption

The absorption of tea catechins occurs mainly in the small intestine and also in the colon, thanks to the metabolism of microbiota. The uptake rate is low, corresponding to about 1.68% in humans, a similar value was found in rats, in comparison with about 14% in mice. The low absorption of tea catechins is partially attributed

to their chemical conformation, which presents a large hydration shell, due to the presence of many hydroxyl groups. In vitro studies on $Caco_2$ cell line models suggest that the behavior of tea catechins in the intestinal epithelium is complex. Transepithelial transport is supposed to occur via paracellular diffusion. However, there are growing hypotheses on the presence of other polarized transport mechanisms for the absorption of tea catechins.

18.3.2 Metabolism

The bioavailability of tea catechins is strictly related to their metabolism at intestinal, microbial, and hepatic levels. In the small intestine, catechins are absorbed as glucuronide and sulfate conjugates, and methylated metabolites, to facilitate their transport to the liver. Catechins and their metabolites present in the intestinal lumen reach the large intestine which are intensely metabolized into small phenolic acids and valerolactones through microbiota activity. In particular, the microbial metabolism can be summarized as follows: (1) the C-ring is opened via the cleavage of an ether bond -O- by hydrogenation, and gallated catechins are degallated; (2) lactones are formed with partial dehydroxylation on the B-ring; (3) phenolic acids and some of their conjugate derivatives are formed; and (4) benzoic acids and some of their conjugates are further formed by oxidation and conjugation. The end metabolites include phenolic acids, benzoic acids, and some of their conjugates.

An in vitro study reported that human serum albumin is able to transport EGCG through the circulation system. Furthermore, this blood protein could prevent the oxidation of EGCG in alkaline conditions, stabilizing it in bloodstream. Indeed, the differences in serum albumin levels in humans may be one of the reasons for the interindividual variability in response to administration of catechins (Ishii et al. 2011).

Catechin metabolites are excreted in feces and urine, through the metabolism in the liver and kidneys, which is less intense than gut metabolism. In more detail, the liver cytosolic catechol-O-methyltransferase quickly catalyzes methylation of the catechins, which decreases their hydrophilicity. Subsequently, sulfurylation and glucuronidation occur in the liver and kidneys to allow the elimination of the methylation products. Another factor that can influence the interindividual variability in response to administration of catechins could be the polymorphisms of catechol-O-methyltransferase.

Several in vitro and in vivo *studies* have allowed a hypothesis of the steps involved in catechin metabolism in humans, as presented in Fig. 4.

As anticipated at the beginning of this section, the human studies on catechin bioavailability yield different and controversial results. In particular, the detected urinary excretion values of catechin metabolites range from traces to about 10% of the ingested amount. However, Del Rio et al. observed that by extending focus from catechin conjugates to include the colonic microflora-derived poly-hydroxyphenyl-g-valerolactones, the urinary excretion reaches 39% of the ingested catechin amount (Del Rio et al. 2010).

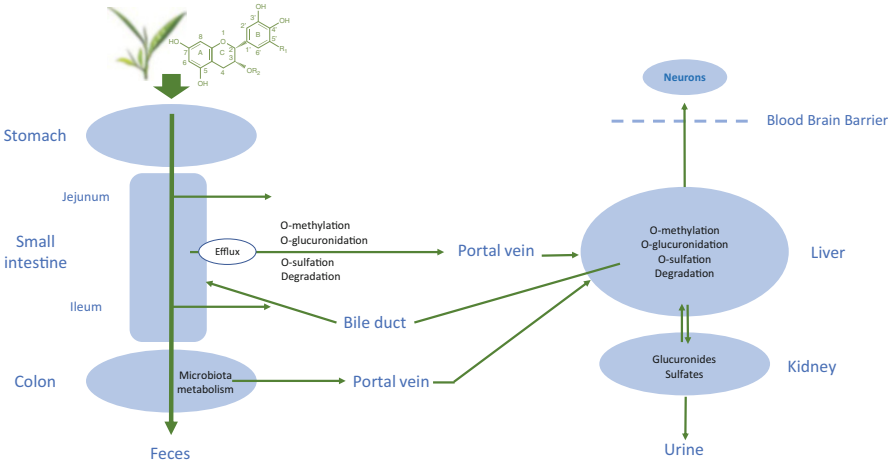


Fig. 4 The proposed metabolism fate of tea catechins in human

18.3.3 Factors Influencing Tea Catechins Bioavailability

Some studies have also assessed the impact of different factors on bioavailability of catechins.

18.3.3.1 Diet

Vitamin C, Omega-3 polyunsaturated fatty acids, and piperine have been found to improve the bioavailability of catechins by preventing their oxidation or glucuronidation. Moreover, clinical studies show that the bioavailability of catechins is enhanced in fasted subjects.

18.3.3.2 Brewing Methods

The use of hard water in tea brewing could affect the bioavailability of catechins due to its higher concentration of metals, especially Ca^{2+} and Mg^{2+} (Ishii et al. 2011).

The results of studies on the bioavailability impact of adding milk in tea beverages, a typical British habit, are unclear. In spite of a relatively recent in vitro study demonstrating that the intestinal absorption of catechins was significantly enhanced in milk added tea, two older human studies showed that adding milk to tea does not increase the bioavailability of tea catechins (Xie et al. 2013).

18.4 Bioactivities

Compounds identified in green tea are involved in several biological activities and can have various health-promoting functions. These include anticancer, antiobesity, and antidiabetic activities, as well as protective effects against cardiovascular disease (CVD) and neurodegenerative disease, and many others (Sanlier

et al. 2018). The therapeutic potential of green tea has been directly linked with its polyphenol content, and particularly for the catechins, which include EGCG as the most abundant, and most investigated, tea catechin (Granja et al. 2017; Pastoriza et al. 2017). There are several mechanisms involved in the protective actions of these phytochemicals. However, as many health disorders are related to oxidative stress and overproduction of inflammatory markers, the protective effects of tea catechins appear to be mainly related to their antioxidant and anti-inflammatory activities.

18.4.1 Neuroprotective Activity

Neurodegenerative diseases are a heterogeneous group of disorders that are characterized by progressive degeneration of the structure and function of the central or peripheral nervous systems (http://www.nature.com/subjects/neurodegenerative-diseases). The progressive degeneration of neurons leads to changes in behavior and cognitive function (e.g., dementia) and to motor dysfunction. Alzheimer's disease constitutes approximately 70% of all dementias, and its incidence increases with age, with a doubling every 5–10 years. Treatments for neurodegenerative diseases represent a great economic burden, and they already contribute US$20 billion to a market that, according to the World Health Organization, is expected to grow and to become the second leading cause of death by 2040, after CVD (Gammon 2014).

Accumulating evidence has implicated oxidative stress in the initiation and progression of such neurodegenerative diseases, and therefore considerable attention is now being paid to the possibility of using nature antioxidants for prevention and treatment. There is an increasing interest in the health properties of tea and a significant rise in scientific studies on the medicinal properties and health benefits of tea (Cooper et al. 2005). Green tea has a better neuroprotective role than other teas from *Camellia sinensis* due to the higher amounts of catechins (phenolic compounds with powerful antioxidant activities) and iron-chelating and anti-inflammatory agents (amazon, Mandel et al. 2012). The mechanisms of action of tea catechins still remain to be fully defined. As well as antioxidant and free radical scavenging effects, their modulation of cell apoptosis and neuroplasticity, attenuation of inflammatory responses, and anti-aggregation effects are likely to have roles here (Sutherland et al. 2016, Ortiz-López et al. 2016).

Some recent studies have shown that catechins from green tea can promote neuroprotection against oxidative and inflammatory stressors in obese rats supplemented with a cafeteria diet (Macedo et al. 2017). The antioxidant properties of green tea are also useful for the protection of memory against deficits due to aging and to brain insults like ischemia reperfusion (Schimidt et al. 2017). Recently, the neuroprotective potential of green tea was also reported in a model of Alzheimer's-like disease (Schimidt et al. 2017).

As well as EGCG, the other major catechins in green tea are: (−)-epicatechin, ECG, and EGC. EGCG is the major catechin isolated from the polyphenolic fraction of green tea, and it can account for 50% to 80% of the catechin content. As well as

its antioxidant properties, many beneficial biological actions of EGCG are known to be mediated through further specific mechanisms, which include its interactions with cell surface membrane proteins and specific receptors and its regulation of signalling pathways and transcription, DNA methylation, mitochondrial function, and cell autophagy (Kim et al. 2014). In vivo studies in mice have demonstrated that EGCG can reduce β-amyloid generation, modulate amyloid precursor cleavage, and reduce cerebral amyloidosis, with inhibition of β-amyloid-induced cognitive dysfunction, through modification of secretase activity (Lee et al. 2009). Aktas and co-workers demonstrated that EGCG mediates T-cell nuclear factor (NF)-κB inhibition and has neuroprotective effects in autoimmune encephalomyelitis, an animal model for multiple sclerosis (Aktas et al. 2004). EGCG can induce down-regulation of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α and can thereby inhibit microglial activation (Anandhan et al. 2013). Islet amyloid polypeptide formation is inhibited by EGCG, and preformed amyloid fibrils are disaggregated (Schrag et al. 1998). Similar to EGCG, catechin has shown neuroprotective properties in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's diseases, via its transitional metal chelating property (iron, copper) and its antioxidant activity (Mandel et al. 2008). Catechin also has anti-inflammatory properties (Hirao et al. 2010). This suggests that catechin might also have a neuroprotective role in brain injuries. In a rat model of middle cerebral artery occlusion, pretreatment with catechin hydrate resulted in neuroprotective effects through NF-κB-mediated downregulation of inflammatory responses (Ashafaq et al. 2012).

18.4.2 Anticancer Activity

In carcinogenesis, critical roles are played by increased levels of reactive oxygen species (ROS), and modulation of oxidative stress (i.e., an imbalance between increased ROS and cell antioxidants) might be the key mechanism through which phytochemicals act in tumorigenesis and cancer chemoprevention (Chikara et al. 2018). ROS include superoxide anion radicals, hydrogen peroxide (H_2O_2), and hydroxyl radicals, and these are generated not only during mitochondrial oxidative phosphorylation but also during inflammation, infection, mechanical and chemical stress, and exposure to ultraviolet and ionizing radiation. Oxidative stress in the first phase of cancer development (i.e., initiation) can lead to mutations in oncogenes and tumor-suppressor genes that are often related to the formation of oxidative stress-associated DNA adducts. In the promotion stage of cancers, oxidation of cysteine and methionine residues result in changes in protein structures and enzymatic activities, which results in dysregulation of signalling pathways and expansion of transformed cells. Furthermore, increased levels of lipid peroxidation products (e.g., 4-hydroxynonenal) that can interact with and modify proteins and DNA have also been shown to be associated with carcinoma progression.

The therapeutic properties of catechins in the prevention and treatment of cancers are mainly attributed to their free-radical scavenging properties and their metal ion

chelation (especially of iron). Several studies have demonstrated the antioxidant capacity of EGCG and shown that EGCG has stronger antiradical activity compared to other green tea catechins and to vitamin E and vitamin C (Granja et al. 2017). However, beyond simple antiradical actions, additional mechanisms appear to be involved in the protective effects of these phytochemicals (Bernatoniene and Kopustinskiene 2018). Green tea catechins can modulate the cellular ROS levels through inhibition of pro-oxidant enzymes, such as cyclooxygenase, inducible nitric oxide synthase, lipoxygenase, and xanthine oxidase, and by induction of the expression of superoxide dismutase, catalase, and glutathione peroxidase, the major enzymes that protect the cell against oxidative damage.

The molecular mechanisms responsible for this induction of antioxidant enzymes by EGCG have been extensively investigated. Catechins, and particularly EGCG, can bind to various molecules and modulate their activities, and thus modulate cell-signalling pathways (Rahmani et al. 2015). It has been shown that EGCG can activate nuclear factor erythroid 2-related factor (Nrf2), which is considered to be an important target for cancer prevention. In this way EGCG can induce expression of antioxidant enzymes. Further, EGCG appears to be involved in phosphorylation of the serine and threonine residues of Nrf2 that are responsible for Nrf2 activation. The effects of tea catechins on lipid peroxidation in liver carcinoma cells were studied. It was reported that EGCG suppressed H_2O_2-mediated cytotoxicity by increasing the levels of cellular glutathione (Chikara et al. 2018). As estrogen is believed to be responsible for breast cancer development, the anti-breast cancer efficacy of tea polyphenols has been ascribed in part to inhibitory activities on the enzymes involved in estrogen biosynthesis (Capellino et al. 2014).

Some studies have also reported that EGCG has protective effects against cancer cells through induction of their cell cycle arrest and apoptosis. Indeed, EGCG has been shown to induce p53-dependent apoptosis in a human lung carcinoma cell line (Yamauchi et al. 2009). Yamauchi et al. (2009) examined cell growth/proliferation and apoptosis with the addition of catechin, epicatechin, ECG, EGC, and EGCG to A549 cells. They showed that at 48 h after treatment, catechin and epicatechin had no suppressive effects on cell viability at up to 100 μmol/L, while ECG had some effect, and EGC and EGCG significantly suppressed cell viability. When a mixture of these compounds was applied, cell viability was decreased even at 10 μmol/L. Also, they evaluated caspase 3/7 activity, which is an indicator of cell apoptosis, and they reported that only EGCG at 100 μmol/L significantly induced caspase 3/7 activity in these A549 cells.

Santos et al. (2018) optimized the formulation of white tea, fermented rooibos, and roasted mate. The antiproliferative activities were evaluated in human hepatoma carcinoma (HepG2) cells and human colorectal adenocarcinoma epithelial (Caco-2) cells. The optimized tea formulation that contained various catechins demonstrated antioxidant effects through its inhibition of ROS generation in the HepG2 cells. In cell viability tests of these tea extracts in this in vitro study, some cytotoxicity against both of these cell lines was seen. The concentration needed to kill 50% of the cells was 500 μg/mL and was similar for both of these cell types.

In another study carried out on the A549 human lung carcinoma cell line, where these cells were treated with EGCG at 10 μmol/L to 100 μmol/L, reduced formation of capillary tube-like structures was observed (Li et al. 2013). They also reported downregulation of expression of some signalling molecules in the presence of EGCG, including hypoxia-inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF). This confirmed the inhibitory effects of EGCG on tumor angiogenesis: the growth of new blood vessels that are needed to ensure nutrient and oxygen supplies to tumor cells.

The efficiency of EGCG for inhibition of metastasis formation has also been investigated. As reported by Takahashi et al. (2014) for human lung cancer cell lines, EGCG inhibited phenotypes of epithelial-mesenchymal transition (EMT) in a dose-dependent manner. Cell invasiveness and the metastatic activity of cancer cells are strongly related to EMT, and EMT activation can be stimulated by a number of biological factors, such as TNF-α and tumor growth factor-β, as well as by mechanical stimuli. They also showed that EGCG can cause alterations to cell membrane organization, which result in inhibition of cell motility and increased cell stiffness.

The chemopreventive potential of green tea catechins has been investigated in several in vivo studies. The prevention of DNA oxidative damage in lymphocytes, colonocytes, and hepatocytes has been reported, where rats were fed a green tea extract for 5 days at the equivalent human dose of 500 mL/day, with no effects seen with the lower dose of 100 mL/day (Kager et al. 2010). The therapeutic activity of EGCG for inhibition of growth and metastases of colon tumors in mice has also been studied. Protective effects of EGCG on preneoplastic lesions in the mouse colon were explained to be due in part to upregulation of the Nrf2-uridine 5′-diphosphate-glucuronosyltransferase 1A (UGT1A) signalling pathway (Yuan et al. 2008). Zhu et al. (2014) demonstrated that skin treatment with EGCG can reduced ROS in skin cells via upregulation of superoxide dismutase 2 and heme oxygenase, which thereby protected the mitochondrial DNA from damage caused by ionizing radiation.

18.4.3 Activity Against Cardiovascular Diseases

Tea consumption has been suggested to be associated with a variety of physiological functions that might be responsible for protection against CVD. CVD is characterized by the development of atherosclerosis, which is an inflammatory condition that is associated with increased levels of ROS and with vascular endothelial dysfunction, accumulation of fatty deposits, oxidation of plasma low-density lipoprotein (LDL), atherosclerotic plaque formation and rupture, platelet aggregation, and obstructions to the arterial blood flow (Santhakumar et al. 2018).

Initially, the reduction of CVD risk by green tea consumption was attributed mainly to the antioxidant and anti-inflammatory properties of the tea catechins. However, recent evidence suggests that the protective role of tea catechins against

CVD appears to be due to their regulation of lipid metabolism, improvements to vascular endothelial dysfunction, effects on cell proliferation, modulation of platelet aggregation, and antithrombotic activity. Animal studies have shown that tea catechins can reduce blood levels of total cholesterol, LDL, and triglyceride and can slow the development and progression of atherosclerosis (Santhakumar et al. 2018).

The modulation by the tea catechins of endothelial cell activation, which contributes to alterations to endothelial function, was evaluated in vitro by Carnevale et al. (2014). In their study, treatment with catechin and EC was accompanied by reduced markers of endothelial cell activation, which was induced by activated platelets from patients with peripheral artery disease. Furthermore, in another study, it was shown that treatment of a human retinal pigment epithelial cell line with EGCG suppressed inflammatory effects induced by TNF-α, including induction of ROS, upregulation of intercellular adhesion molecule (ICAM-1) expression, and increased monocyte adhesion to retinal pigment epithelial cells. These data indicated that this TNF-α inhibition was mediated via the NF-κB pathway (Thichanpiang and Wongprasert 2015). In another in vitro study on human neutrophils, a catechins mixture (including EC, ECG, EGC, EGCG) suppressed the release of pro-inflammatory cytokines, and also the expression of NF-κB and myeloperoxidase activity, and the production of HOCl (Marinovic et al. 2015). Decreased ROS levels were ascribed to increases in antioxidant enzyme activities, which they attributed to induction by these catechins. They also reported increases in phagocytic activity of the neutrophils.

Gallate is a pyrogallol moiety that can act as a strong antioxidant. It is esterified at position 3′ on the C-ring of EGCG, GCG, and ECG, and it enhances the antiplatelet and antithrombotic activities of these phytochemicals, compared to their counterparts, EGC, GC, EC, respectively, and to catechin. This confirmed that their antioxidant activities might be involved here, although not as a determining factor. However, the antiplatelet and antithrombotic activities of these catechins can be attributed to their modulation of various cellular targets. ECCG can suppress collagen-induced platelet aggregation by inhibition of arachidonic acid liberation from membrane phospholipids (Jin et al. 2008).

Epigallocatechin-3-gallate has been shown to reduce myocardial damage induced by cigarette smoke in rats (Gokulakrisnan et al. 2011). The protective role of this in vivo EGCG treatment was confirmed by decreased pro-inflammatory markers, which have a key role in atherosclerosis, such as NF-κB, TNF-α, cyclooxygenase-2, and inducible nitric oxide synthase in the cardiac tissue of the rats. Furthermore, the EGCG treatments resulted in fewer lipidemic pathologies (i.e., high cholesterol, fatty acids, triacylglycerols) and decreased lipid-metabolizing enzyme activities in the serum and cardiac tissue (Gokulakrisnan et al. 2011). In a study in mice, EGCG administration (40 mg/kg/day for 18 days) significantly reduced the size of atherosclerotic plaques in the aortas, and increased high-density lipoprotein (HDL), decreased LDL, and had little effect on triglycerides in plasma. Also, EGCG significantly inhibited hepatic lipid accumulation and suppressed inflammatory processes, whereby across six cytokines examined, the levels of TNF-α and

interleukin (IL)-6 were reduced, while those of IL-10 were increased (Wang et al. 2018a). As they surmised, the increased IL-10 might promote anti-inflammatory conditions and thus protect against the development of atherosclerosis. In the same study, it was suggested that EGCG eventually activated gene expression to promote hypolipidemic effects.

18.4.4 Antidiabetic Activity

It has been demonstrated that in diabetes prevention and treatment, tea catechins can be beneficial through a number of actions, including regulation of insulin secretion of pancreatic β-cells, control of blood glucose levels, inhibition of insulin resistance, and control of inflammation markers (i.e., expression of pro-inflammatory cytokines) and oxidative status (Granja et al. 2017). The protective actions of these phytochemicals in diabetes are regulated through various mechanisms. Studies on pancreatic β-cell lines showed that EGCG improves glucose-stimulated insulin secretion through stimulation of insulin receptor substrate-2 signalling. It has also been suggested that EGCG administration can significantly reduce glucose levels by decreasing the formation of "advanced glycation end products" (AGEs), whereby AGEs formation is suppressed via the regulation of Nrf2 function (Sampath et al. 2017). Therapeutic properties have also been ascribed to the inhibitory activities of the tea catechins on α-amylase and α-glucosidase. An in vitro study showed that tea catechins and EGCG alone more strongly inhibited α-glucosidase than α-amylase. Based on half maximal inhibitory concentration, tea catechins showed up to 1000-fold the efficacy of the antidiabetic drug acarbose (Gao et al. 2013). In an in vivo study of sucrose loading in rats, Satoh et al. (2015) demonstrated that an aqueous extract of tea leaves resulted in dose-dependent lowering of plasma glucose, with no effects on insulin levels. In the same study, they showed inhibition of α-glucosidase and α-amylase by this treatment.

An investigation carried out by Alves et al. (2015) showed that in prediabetic rats, 2 months consumption of white tea improved their overall metabolic status. Here, as well as higher insulin sensitivity and lower glucose intolerance, they also showed improved protein oxidation status for these rats. A similar in vivo study where diabetic mice were fed with a green tea extract that contained high levels of EGCG (>94% EGCG; <5% other catechins) confirmed these pronounced anti-diabetic effects of these phytochemicals (Ortsäter et al. 2012). Their data also indicated that EGCG exerts its antidiabetic activity through reduction of insulin resistance and preservation of pancreatic β-cells. EGCG treatment resulted in fewer islets of Langerhans that showed pathological changes. There was also a reduction in the levels of the oxidative stress markers, and this was linked to the antioxidant effects of EGCG. Thus, they suggested that EGCG is a potential therapeutic agent for prevention and treatment of diabetes mellitus. In addition, Ahmad et al. (2015) reported that in rats the interventions with catechins and EGCG resulted in significantly lower serum glucose and lipid levels (i.e., cholesterol, LDL) than those that received a high cholesterol and sucrose diet. Here, EGCG was more effective

against hyperglycemia than the other catechins, which reduced hypercholesterolemia more effectively.

Epigallocatechin-3-gallate has been shown to provide this beneficial modulation of glucose and lipid metabolism through downregulation of genes involved in gluconeogenesis and synthesis of fatty acids, triacylglycerol, and cholesterol. Studies provided evidence that green tea catechins change the redox state of the cells and affect the activity of several kinases involved in gluconeogenesis (Granja et al. 2017). An in vitro study by Yasui et al. (2010) provided evidence that in H4IIE rat hepatoma cells, green tea catechins can decrease gene expression of the glucose-6-phosphatase and phosphoenolpyruvate carboxykinase.

Chronically elevated levels of free fatty acids in plasma may also have a profound role in insulin resistance. Indeed, treatment with EGCG and ECG in a mouse skeletal muscle cell model improved free fatty acid-induced insulin resistance through suppression of phosphorylation of serine 307 in insulin receptor substrate-1, with regulation of the protein kinase activation pathway in dose- and time-dependent manners (Deng et al. 2012).

18.4.5 Anti-obesity Activity

Investigations have demonstrated that tea catechins have potent efficiency in obesity treatments, via a direct impact on adipose tissue, and that they can promote decreased adipocyte differentiation. It has been shown that these phytochemicals have suppressive effects on the enzymes involved in the synthesis of fatty acids, triacylglycerols, and cholesterol, and on the other hand, tea catechins have been shown to stimulate the enzymes involved in fatty acid mobilization (Suzuki et al. 2016).

In rats induced to obesity, Rocha et al. (2016) demonstrated that daily consumption of green tea extract (500 mg/kg body weight) significantly inhibited the gain in body weight (by 24% versus control), decreased adipose tissue (i.e., subcutaneous, retroperitoneal fat) and adiposity index, reduced hypertrophy of adipocytes, and decreased triacylglycerols and cholesterol contents in blood and liver tissue. Increased levels of adiponectin in the plasma after consumption of green tea extract suggest that green tea catechins can also promote secretion of this adipokine by adipocytes. It has also been reported that treatment with green tea catechins decreases plasma levels of the inflammatory cytokines, such as IL-6 and TNF-α (Rocha et al. 2016). However, in the course of this green tea treatment, the biochemical parameters of uric acid and creatinine did not differ significantly among the treatment groups (obese rats, without and with green tea consumption), which suggested that this green tea consumption did not cause any kidney or liver damage. In the same study with obese rats, green tea modulated gene expression of key proteins involved in lipid metabolism in their adipose tissue. This was seen as a significant decrease in adipocyte differentiation and downregulation of mRNA expression of several lipogenic enzymes: fatty acid synthase, pyruvate dehydrogenase, stearoyl-CoA desaturase 1, and diacylglycerol acyltransferase. On the other

hand, mRNA expression levels of the main lipolytic enzymes (i.e., hormone-sensitive lipase, adipocyte triacylglycerol lipase, lipoprotein lipase) and the protein perilipin were significantly increased (Rocha et al. 2016). According to the so-called AMP-activated protein kinase (AMPK) hypothesis, it has been postulated that the effects of green tea catechins on genes are mediated through AMPK, which is an enzyme that has a key role in downregulation and upregulation of the expression of various genes (Yang et al. 2016). Catechins are known to activate AMPK, which can result in inhibition of the expression of the lipogenic enzymes, and suppressed differentiation of adipocytes.

Other studies have suggested that green tea catechins can interfere with the emulsification of lipids, which leads to suppressed fat digestion. This might thus be an additional way in which these phytochemicals influence obesity. It has been proposed that this interference is due to increased particle size and decreasing surface area of the emulsified lipids and that these changes will influence lipase activity and slow down lipid hydrolysis. A study performed with green, white, and black tea infusions showed that white tea was more effective in inhibition of pancreatic lipase activity in vitro than green tea, with half-maximal concentrations for inhibition of 22 μg gallic acid equivalents (GAE)/mL and 35 μg GAE/mL, respectively. Here, black tea was inactive even at 200 μg GAE/mL (Gondoin et al. 2010). The inhibitory effects of these catechins were ascribed to the formation of a complex with the lipase, which modifies its conformation and leads to decreased catalytic activity.

Green tea catechins can also stimulate thermogenesis through inhibition of the enzymes that metabolize noradrenaline. This has been suggested as an additional mechanism by which catechins regulate the balance between lipid anabolism and catabolism and increase energy expenditure. In addition, tea catechins have been shown to inhibit starch digestion by inhibition of α-amylase. In vitro inhibitory activities against pancreatic α-amylase of EGCG and oolong tea were indeed confirmed, with half-maximal inhibitory concentrations of 0.350 mg/mL and 0.375 mg/mL, respectively (Fei et al. 2014).

18.4.6 Other Activities

Other health-promoting effects of tea catechins have also been described, such as anti-inflammatory, antiviral, antibacterial, antifungal, anti-osteoporosis, anti-allergenic, anti-estrogenic, and photoprotective activities. As reviewed by Siddiqui et al. (2016), tea can inhibit growth and multiplication activity of different bacteria, such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Helicobacter pylori*. Activities against human pathogenic yeast, such as *Candida albicans*, have also been reported, with these effects not being lethal against intestinal microbiota. Furthermore, beneficial effects for *Bifidobacterium* spp. have also been reported for tea.

Several mechanisms have been proposed for the actions of tea catechins against viruses: antioxidant activity, enzyme inactivation, cell membrane disruption, prevention of viral attachment and penetration into cells, and induction of the self-defense mechanisms of the target cells. The efficiency of the antiviral activity against influenza virus infection in chicken was confirmed (Lee et al. 2012), and EGCG was shown to inhibit in vitro entry of Zika virus into host cells (Sharma et al. 2017). Furthermore, EGCG is involved in dose-dependent mechanisms that inhibit HIV-1 infectivity of human T cells and macrophages, whereby at physiological concentrations of EGCG (<10 μmol/L) it significantly inhibited HIV-1 p24 antigen production (Nance et al. 2009). For this inhibition of HIV, the green tea catechin appears to act by adhering to the target cells, which prevents the virus from anchoring and entering the host. As EGC, ECG, and catechin were ineffective here, this indicated that both the pyrogallol and galloyl groups are required for this activity. Furthermore, evaluation of cytotoxicity in HIV-1-infected cell cultures demonstrated that at physiological concentrations, EGCG did not significantly inhibit cell viability, proliferation, and apoptosis. Inhibitory activities of EGCG against other viruses have also been confirmed, such as against hepatitis C virus, adenovirus, and Herpes simplex virus (Pastoriza et al. 2017).

Tea catechins can protect bone health and modulate the regulation of bone formation and loss. Studies carried out on cell lines and in animal models have shown positive effects of tea catechins on osteoporosis. As reviewed by Đudarić et al. (2015), EGCG can influence the signalling pathways involved in osteoblastogenesis and osteoclastogenesis, which in rodents results in increased bone mineralization, inhibition of osteoclast differentiation, and decreased formation of calcium stone deposits. This protective role of tea catechins in bone health has again been ascribed to their antioxidant properties and their reduction of the production of inflammatory mediators.

Wang et al. (2018b) showed recently that when rats with diabetic retinopathy were treated with catechins by intravitreal injection (50–200 mg/kg/day) for 8 weeks, there was inhibition of activation of NF-κB and decreased production of inflammatory factors (i.e., IL-1, IL-6, TNF-α), which weakened the pathological changes in the retinal tissues. Furthermore, as reviewed by Rahman et al. (2018), green tea polyphenols also have significant roles in the treatment of inflammatory bowel disease, through their regulation of Toll-like receptor 4, which blocks NF-κB activation, and thus inhibits TNF-α production.

18.5 Benefits

Many clinical studies have been carried out to examine the health-promoting functions of green tea catechins. A large number of studies have shown that green tea consumption is associated with health benefits, with confirmation of the protective role and therapeutic potential of tea catechins in the treatment of several diseases.

18.5.1 Neurodegenerative Diseases

Due to their antioxidant activities and anti-amyloidogenic properties, green tea catechins are being investigated as dietary phytochemicals for their beneficial effects in neurodegenerative diseases (Rigacci and Stefani 2015). We previously evaluated the brain accessibility of EGCG in relation to its potential neuroprotective effects (Pogačnik et al. 2016). Primary cortical neuron cultures were exposed to oxidative insult in the absence and presence of the catechins, and neuroprotection was assessed through evaluation of apoptotic-like and necrotic-like cell death. EGCG rapidly crossed the blood-brain barrier model, and in doing so, it protected against oxidation-induced neuronal necrotic-like cell death by ~40% and in apoptosis by ~30%. Also, EGCG effectively inhibited α-synuclein fibrillation over the relevant timescale. Based on the effects of the catechins studied, we concluded that EGCG is the most promising neuroprotective compound (Pogačnik et al. 2016).

18.5.2 Cancers

It has been suggested that a diet rich in tea catechins is inversely associated with incidence of a variety of cancers (Chikara et al. 2018). Diet enriched with green tea led to significant reduction in urinary 8-hydroxy-2′-deoxygunosine, which is an oxidative stress-related DNA adduct that has been associated with precancerous and cancerous tissues. Consumption of green tea catechins (1.3 g/day for 6 weeks) in a phase II clinical trial with men suffering from prostate cancer resulted in reduced serum levels of hepatocyte growth factor (HGF, a prostate-specific antigen) and VEGF (an angiogenic stimulus for tumors) (McLarty et al. 2009).

Beneficial effects of green tea on ovarian cancer were ascribed to induction of apoptosis and to suppression of expression of proteins involved in inflammation, cell motility, and angiogenesis. Studies have shown that in premenopausal and postmenopausal women, green tea consumption can decrease serum estrogen levels (as a risk factor for ovarian cancer) potentially through modification of estrogen metabolism or conjugation (Fuhrman et al. 2013). Black tea consumption has been shown to both increase and decrease (Sanlier et al. 2018) ovarian cancer risk.

Zhang et al. (2012) tested the activity of EGCG supplementation on parameters related to cell proliferation, invasion, and angiogenesis in patients with breast cancer undergoing radiotherapy treatment. In comparison to those receiving radiotherapy alone, the serum levels of VEGF and HGF were significantly lower with radiotherapy plus EGCG (400 mg, for 2–8 weeks). Then in the same study, in vitro treatment of cancer cell lines with EGCG resulted in cell cycle arrest, suppression of cell proliferation and invasion, reduction of activation of metalloproteinase-9 and metalloproteinase-2, and reduction of NF-κB.

As reviewed by Sanlier et al. (2018), some studies have reported no correlations between green tea consumption and cancer risk, while others have reported significant positive correlations, or that green tea consumption significantly effects its protective actions. According to a recent systematic review of 11 cohort studies, 9

case-control studies, and 1 clinical trial, it cannot be concluded that green tea intake can reduce incidence of breast cancer (Najafi et al. 2018). Guo and co-workers (2017) carried out a dose-response meta-analysis and concluded that high doses of tea consumption (7 cups/day) reduced the risk of prostate cancer. Then an inverse association between tea consumption and skin cancer risk was reported in a case-control study carried out by Rees et al. (2007), while Asgari et al. (2011) reported that there was no such association. In a further dose-response analysis, here of observational studies, it was suggested that green tea consumption decreased the risk of lung cancer, while black tea consumption did not have any effects (Wang et al. 2012).

A large prospective cohort study was performed over 15 years in China on 165,000 adult male participants aged >40 years and free of pre-existing diseases (Liu et al. 2016). This showed that regular green tea consumption was associated with reduced risk of all-cause mortality, and of CVD-specific and cancer-specific mortality, compared to those who did not drink green tea. For non-smokers, non-regular alcohol drinkers, and rural residents, this inverse association for green tea consumption was further strengthened. Indeed, these data for all-cause and CVD mortality are in line with most previous studies. However, the observations for cancer mortality are not consistent. The results from a meta-analysis of 11 prospective cohort studies that focused on cancer mortality indicated that green tea consumption was not associated with cancer mortality, while black tea consumption was shown to be inversely associated with all-cancer mortality (Tang et al. 2015). Finally here, other studies have reported no associations between green tea consumption and cancer mortality (Kuriyama 2008).

18.5.3 Cardiovascular Diseases

High incidence of CVD has an enormous socioeconomic impact and is associated with incorrect diet and lifestyle. Several epidemiological studies have investigated the relationships between tea consumption and the development of CVD. Generally, it has been indicated that tea catechins are protective against CVD through actions on a number of different systems, including regulation of intestinal absorption and serum levels of cholesterol; activation of antioxidant and inhibition of pro-oxidant enzymes; improvements to vascular endothelial function; suppression of platelet adhesion; reduction of the risk of inflammation and oxidative damage; antithrombotic activity; regulation of the blood lipid profile; and lowering of blood pressure (Sanlier et al. 2018). Indeed, Jowko with co-workers (2015) showed that supplementation with a green tea extract (980 mg polyphenols daily for 4 weeks) protected against exercise-induced oxidative stress. In their randomized, double-blind, placebo-controlled study, there were higher total polyphenols and total antioxidant capacity in the blood plasma of male sprinters before a sprint, with lower plasma values of the oxidation product malondialdehyde after the sprint. At the same time, there were no effects on the muscle damage marker creatine kinase compared to placebo (i.e., no green tea consumption). Furthermore, a double-

blind clinical study on 60 mildly hypercholesterolemic subjects showed that drinking catechin-enriched green tea (as 781 mg catechin daily) or oolong tea (640 mg catechin daily) for 12 weeks provided hepato-protection (Venkatakrishnan et al. 2018). Here, significance was seen for increased expression of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase), as well as decreased body weight and plasma lipid profile.

A population-based prospective cohort study on 40,530 people showed that green tea consumption was inversely associated with all-cause mortality, with a stronger inverse association with CVD mortality, and stroke mortality in particular (Kuriyama 2008). These inverse associations were more pronounced in women. Consumption of 500 mL green tea per day compared to 100 mL/day reduced the risk of CVD death by 31%. This was particularly seen for cerebral infarction, whereby the women who consumed 500 mL green tea per day had 62% lower risk from cerebral infarction compared to those consuming 100 mL/day. Also, a meta-analysis of prospective observational studies defined protective effects against CVD for increased green tea consumption (Pang et al. 2016).

In contrast, several studies have failed to show favorable effects of green tea consumption against CVD. In an earlier Japanese study, there was no association seen between tea consumption and CVD in men, while in women, there was an almost 40% lower risk (Mineharu et al. 2011). Ikeda and co-workers carried out a prospective cohort study that included almost 30,000 men and women aged 40–69 years and evaluated the association between plasma levels of the tea catechins (i.e., epicatechin, ECG, EGC, EGCG, total) and incidence of stroke or coronary heart disease (Ikeda et al. 2018). They found no significant associations. However, when the analysis was stratified by smoking status, positive correlation was found between plasma levels of EGCG and reduced risk of stroke for the group of male non-smokers.

18.5.4 Diabetes

Green tea has been recognized as a potential nutritional strategy associated with positive effects for prevention and control of diabetes mellitus. It has been shown that catechins can control hyperglycemia and reduce complications associated with type 2 diabetes mellitus (Alipour et al. 2018). Indeed, a double-blind controlled study on 52 patients with type 2 diabetes showed that 12 weeks of 583 mg green tea catechins per day significantly increased insulin levels, whereas no significant difference was seen for glucose levels and glycated hemoglobin (Nagao et al. 2009). Furthermore, meta-analysis of 22 randomized controlled trials that included 1,584 subjects revealed that consumption of green tea catechins decreased fasting blood glucose (Zheng et al. 2013). The results of subgroup analyses here suggested that the beneficial effects were more pronounced when the green tea catechins consumption was longer than 12 weeks. However, as was indicated, the limited data available did not support a positive effect on fasting blood insulin and glycated hemoglobin levels. Another meta-analysis confirmed that daily consumption of tea

(>3 cups) reduced the risk of type 2 diabetes (Yang et al. 2014). Here, they also noted differences between different regions around the world and differences with regard to sex. Significant association between tea consumption and diabetes risk was shown for an Asian population, but not for the European and American populations. This was explained on the basis that green tea is mainly consumed in Asia, while black tea is preferred in European countries and the USA.

In a randomized controlled crossover trial of overweight male subjects, no effects were seen on serum glucose, insulin, cholesterol, and triacylglycerol levels after 6-week administration of 400 mg (twice daily) decaffeinated green tea, despite significantly increased levels of serum EGCG and urinary EGC and its metabolites (Brown et al. 2011). Another randomized, placebo-controlled crossover study showed that a green tea extract (EGCG, 257.6 mg) ingested with a test meal limited digestion and absorption of starch in humans without causing any side effects, as estimated by $13CO_2$ excretion in the breath (Lochocka et al. 2015). This finding demonstrated that tea catechins can act as inhibitors of glucoside hydrolase enzymes, and it suggested their use as viable alternatives to pharmaceuticals for weight control and treatment of type 2 diabetes mellitus.

18.5.5 Obesity

As reviewed by Sanlier et al. (2018), there have been several epidemiological studies that have confirmed that green tea consumption interferes with dietary lipid absorption and digestion, and thus lowers lipid levels in the blood and lipid accumulation in the tissues. It has been shown that green tea catechins have inhibitory effects on expression of genes for proteins involved in adipogenesis and lipogenesis, and these effects of tea on fat metabolism can be highly relevant for weight management protocols. Indeed, different studies have shown beneficial effects of exercise-induced anti-obesity treatments (e.g., abdominal fat loss, decreased plasma lipid levels, increased lean body mass, and muscle strength) that are enhanced when combined with green tea consumption (Suzuki et al. 2016). An epidemiological study conducted on 6,472 women and men in the United States reported that black, oolong, and green tea consumption was inversely associated with body mass index and other markers of metabolic syndrome (Vernarelli and Lambert 2011). In this study, inflammatory markers (e.g., serum levels of C-reactive protein) were lower in tea consumers of both sexes, with higher HDL-cholesterol for the male tea consumers, and for both the female and male tea consumers, there was no association with tea consumption for LDL-cholesterol and total cholesterol.

Many randomized, placebo-controlled clinical studies conducted on obese subjects with metabolic syndrome showed significant reduction of abdominal fat, waist circumference, body weight, body mass index, LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio, and oxidative stress markers (i.e., malondialdehyde, hydroxynonenal, myeloperoxidase, oxidized LDL) in the subjects who consume daily green tea catechins, compared to the controls (Suzuki et al. 2016). In a randomized, double-blind, placebo-controlled clinical study, 115 women received

high-dose green tea extract for 12 weeks. The results here showed significant weight loss and decreased waist circumference and serum levels of total cholesterol and LDL-cholesterol (Chen et al. 2016). Another clinical trial showed that 12 weeks consumption of a catechins-rich beverage (583 mg green tea catechins per day) for patients with type 2 diabetes resulted in decreased waist circumference and increased adiponectin levels, which is negatively related to visceral adiposity (Nagao et al. 2009). The therapeutic effects of drinking black tea in terms of inhibition of fat absorption were examined in a randomized, placebo-controlled, double-blind cross-over study, where 10-day consumption of 55 mg black tea polyphenols three times per day resulted in increased fecal lipid excretion (Ashigai et al. 2016). As examined recently in a pooled analysis of six human trials, continual consumption of green tea (as a beverage with 540–588 mg catechins) significantly reduced abdominal fat (total, visceral, subcutaneous fat) and improved blood pressure, body weight, body mass index, and waist circumference (Hibi et al. 2018). These observations suggested a protective role of the tea catechins in the development of diseases associated with metabolic syndrome disorders.

However, there have also been indications that have not supported anti-obesity effects of these tea catechins. For example, a cross-sectional survey of 554 participants in Japan did not find any correlation between tea consumption (even for those drinking >4 cups/day) and metabolic syndrome disorders (e.g., increased weight and waist circumference, hypertension, elevated serum triglycerides) (Takami et al. 2013). They proposed some possible explanations for this lack of correlation, such as small variations in the green tea consumption among these individuals, different dietary habits, presence of chronic diseases, lack of information on added sugar, milk, or cream, and the relatively small number of participants. Also, a randomized, double-blind, placebo-controlled trial conducted over 12 weeks on 83 obese premenopausal women who received 300 mg EGCG/day showed no significant effects on body weight, adiposity, energy expenditure, blood lipid levels, and C-reactive protein (Mielgo-Ayuso et al. 2014). However, the EGCG intake had no adverse effects on liver function markers. A similar trial of 937 healthy or obese postmenopausal women who consumed a green tea extract (843 mg EGCG/day) also did not show any changes in body weight, body mass index, or waist circumference after 12 months, and with no changes seen for serum levels of leptin, ghrelin, or adiponectin (Dostal et al. 2016). Finally here, as shown in a randomized clinical trial that included 63 patients with type 2 diabetes, drinking four cups of green tea/day resulted in significant decrease in body weight and systolic blood pressure but no significant changes in blood lipid profile and oxidative stress parameters (i.e., malondialdehyde level) (Mousavi et al. 2013).

18.5.6 Osteoporosis

Several studies on bone health in postmenopausal women showed that green tea consumption increased bone formation, decreased deterioration of bone microstructure, and improved bone strength and quality (Pastoriza et al. 2017). A

systematic review of 11 cross-sectional studies, 4 case-control studies, and 2 prospective cohort studies suggested that tea consumption can reduce the risk of osteoporosis (Sun et al. 2017). In another meta-analysis that included eight cross-sectional studies, four cohort studies, and one case-control study, tea consumption was shown to be beneficial for bone mineral density and to prevent bone loss (Zhang et al. 2017).

18.6 Applications in Food

At present, the importance of food naturalness continues to grow with consumers. For most consumers in developed countries, it is very important that food products be natural. A 2017 review, aiming to draw up the concept of "natural food" held by consumers, shows the importance of freedom from synthetic additives, preservatives, or other artificial ingredients for the perceived naturalness of foods. These consumer expectations represent a big challenge for the food industry and are currently at the forefront of the search for natural-based substitutes for food additives of synthetic origin.

In the last few years, the isolation of catechins from tea has allowed the use of these compounds in food technology, as antioxidants, preservatives, coloring, or flavoring agents. Furthermore, isolated catechins are employed as bioactive ingredients in food supplements, pharmaceutical, and cosmetic products.

The employment of tea catechins in food technology is based on their physical and chemical properties, among which the most important are:

(a) Ability to form precipitates.
As shown in Sect. 2, catechins have the ability to form precipitates, interacting with proteins, but also caffeine. This phenomenon is called *creaming*. The inhibition of the activity of some enzymes, such as lipase, lipoxygenase, amylase, pepsin, and trypsin, attributed to catechins, derives from their ability to form precipitates with protein, as the precipitating enzymes are inactivated. EGCG and ECG contain ester bonds, so have shown a greater ability to form precipitates than other catechins. The precipitation of catechins is also used in the catechin isolation process, which is explained in more detail in the following section.

(b) Scavenger ability.
The antioxidant ability of catechins is well-known. This property is due to the presence of hydroxyl groups in the catechin structure, which can scavenge reactive oxygen species (ROS). Moreover, catechins can trap peroxyl radicals, suppressing free radical chain reactions and terminating the lipid peroxidation process. EGCG and ECG are shown to be the most effective catechins in preventing lipid peroxidation, followed by EGC and EC. Food technologists take advantage of these catechin properties, especially in foods with high fat content or oils.

On the other hand, some precautions must be taken in employing tea catechins in food technology, on the basis of other physical and chemical properties such as:

(c) Temperature and pH sensibility.
As explained above, catechins are unstable at high temperature and pH values. Briefly, when the temperature exceeds 95 °C, EGCG, EGC, ECG, and, EC epimerize into their respective nonepistructured forms (GCG, GC, CG, C). Moreover, catechins are stable in acidic conditions (pH < 4), but when pH values rise above 4, the stability of catechins decreases, and when exceeding a pH value of 8, catechins become extremely unstable.

(d) Oxidizing enzymes sensibility.
The production processes involved in producing the various types of tea are based in part on the sensitivity of catechins to oxidizing enzymes, such as polyphenol oxidase and peroxidase, that oxidize catechins in theaflavins and thearubigins. However, the adjustment of temperature and pH values can contribute to the reduction of oxidizing enzyme activity. In fact, the optimum conditions for their activity are at a temperature of 40 °C and pH of 5.5.

(e) Ion-binding ability.
Catechins are able to bind iron, due to their galloyl group. When this phenomenon occurs in foods, it inhibits iron absorption in the body. Nevertheless, the ion-binding ability of catechins could be useful in the prevention of oxidative damage in foods. Indeed, catechins are able to chelate iron and copper, two metals that catalyze the lipid peroxidation reaction, inhibiting the initiation and propagation phases.

(f) Bitterness.
Catechins are characterized by a bitter taste which is a limiting factor for their use in food technology, as recent studies have shown that bitter taste is one of the main reasons for the rejection of food products by consumers. So, a balance between the active dose of catechins and bitterness is need.

18.6.1 Tea Catechins Isolation

As explained in more detail above, tea leaves contain a wide variety of soluble chemical compounds such as catechins, caffeine, organic acids, chlorophylls, theanine, and others, of which catechins are present in largest amounts. However, the isolation of tea catechins can be difficult due to their nonsignificant difference in solubility or molecular size, and for this reason several isolation methods have been developed. Green tea is considered to be the better starting matrix for catechin extraction and isolation, over other teas, due to its higher concentration of catechins.

Generally speaking, there are three types of catechin isolates reported below.

- The *crude tea extract* contains catechins together with other tea compounds, e.g., caffeine, chlorophyll, and theanine. The production process of crude tea extract involves three main steps: (1) the extraction of soluble compounds into a tea

infusion, (2) the concentration of the obtained solution, and (3) the drying process.

Because of its content of other tea compounds, crude tea extract might be inconvenient for use in foods. For example, the yellowing color of the extract could give an unwanted color to certain foods. Moreover, caffeine could give an unwelcome bitter taste.

- The *catechin mixture extract* contains a high-purity catechin mixture. The crucial step in the production of catechin mixture extract is the decaffeination process, starting from crude tea extract or directly from a tea infusion. Tea leaves themselves are characterized by a high caffeine content, of about 5% d.w., even more than coffee which contains about 1.5% of caffeine d.w. The caffeine content of tea extracts might not be agreeable to consumers, in addition to contributing to creaming phenomena, which makes catechins unstable in aqueous solution.

  As shown in the previous subsection dedicated to this process, several methods are used for tea decaffeination, including organic solvent extraction, synthetic resin absorption, and supercritical fluid extraction. Nowadays, while the extraction of caffeine through organic solvents is very effective, the risk of solvent residuals remaining in the final product has become almost unacceptable. Thus, supercritical fluid extraction and synthetic resin absorption are the most used techniques, despite high setup costs.
- The *individual catechin extract* contains a high concentration of a single type of catechin and is widely used in food supplements or as food-preserving agent, especially in the case of EGCG extract. The isolation of individual catechins represents a big challenge, because of their similar chemical conformation and molecular weight. To approach this critical issue, countercurrent and column chromatography methods have been developed. These two separation methods are based on the differences between catechins in their affinity to solvents and column materials, used in countercurrent chromatography and in column chromatography, respectively.

18.6.2 Tea Catechins as Food Additives

As discussed at the beginning of this section, in addition to their employ in food supplements and fortified foods, tea catechins are used as replacements for synthetic food additives. In particular, tea catechins are used as antioxidants, preservatives, coloring, and flavoring agents.

18.6.2.1 Antioxidant Agents

Catechins are used in foods as antioxidant agents, especially in meat and fish products, due to their ability in preventing lipid peroxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ), are commonly used in food. It has been shown that a chronic consumption of these synthetic compounds, even if in small doses, could have a toxic effect in the body, especially in infants and children.

Some studies have elucidated the effectiveness of catechins in preventing lipid peroxidation, comparing results with those obtained from commonly used antioxidants. Catechins, especially EGCG, are 20 times more active than vitamin E and 4 times more active than BHA. In other studies, catechins have shown a synergic effect with antioxidant agents, such as vitamin C. The greater the level of unsaturation of fats, the more the foods containing them are subject to peroxidation. For example, vegetable oil and fish oil are more susceptible to lipid peroxidation than meat. The use of tea catechins to prevent lipid peroxidation shows positive results in meat (catechin dose used: 300 mg/kg), fish (catechin dose used: 250 mg/kg), and soybean oil (catechin dose used: 40 mg/kg) (Vuong et al. 2011).

18.6.2.2 Preservative Agents

In addition to preventing lipid peroxidation, which leads to food spoilage and lowers shelf life, tea catechins have shown inhibitory activity against microbial growth. Hara-Kudo and colleagues have shown that a crude catechin extract could exert a significant effect on the growth, toxin production, and spore germination of certain species of bacteria. In particular, a crude catechin extract containing a catechin concentration of about 250 μg/mL was found to inhibit the spore germination and vegetative growth of *Clostridium botulinum* and *Bacillus cereus*, whereas *Escherichia coli* O157 serotype appears to be more resistant. Nevertheless, a crude catechin extract containing a catechin concentration of about 500 μg/mL is able to inhibit the vegetative growth and the production of Vero cytotoxins of *Escherichia coli* O157. The mechanisms proposed by the authors for the inhibition of spore germination from catechins are (1) damage to spore membranes, observed through fluorescence microscopy, and (2) interaction between catechins and calcium-binding proteins on the spore surface, hypothesized by authors. These proteins, which allow the cellular absorption of calcium, are in fact fundamental in the germination phase of the spores (Hara-Kudo et al. 2005).

18.6.2.3 Coloring and Flavoring Agents

Some studies have shown the potential effects of tea catechins as coloring and flavoring agents. However, of these, catechins provide contradicting results in terms of their coloring activity. Tang et al. showed that the addition of tea catechins at 200 mg/kg and 300 mg/kg to beef improved color stability and discoloration, respectively (Tang et al. 2006). The mechanism of action proposed by Tang and colleagues was that catechins maintained oxymyoglobin levels during refrigerated storage, retaining the bright red color of meat, and inhibited metmyoglobin production, which confers the brown color to meat. Meanwhile, Martinez et al., using the same concentration of tea catechins on pork meat, found no color-preserving effect, hypothesizing that catechins could actually have had a negative effect on color (Martínez et al. 2005). This meat discoloration could be related to the ability of catechins to bind the iron present in myoglobin. A more recent study, on the other hand, found improved conditions for the stabilization of meat color in the application of a mixture composed by catechins (3 to 19%), carnosine (78 to 94%), and α-tocopherol (0 to 12%) (Liu et al. 2010). The reason for this discrepancy in the results

obtained in studies of the coloring properties of catechins may be ascribed to variability in the experimental conditions.

The flavoring properties of tea catechins have been demonstrated in meat products at a concentration of 200 mg/kg and 300 mg/kg, inhibiting the off-flavors formed during food storage. This effect is ascribed to both the antioxidant and the antimicrobial properties of tea catechins (Martínez et al. 2005).

18.6.3 Tea Catechins Regulatory Status

Despite it having been well established that tea catechins could be used in food supplements as components of *C. sinensis* extract, the community regulations of the various countries are not harmonized regarding the use of tea catechins alone as a bioactive ingredient in food supplement, as is often the case. This occurs because the safety of tea catechins has not yet been clarified. For example, at the European level, the European Commission has asked EFSA to perform this evaluation, and the EFSA scientific opinion was published in 2018. This document established the safe consumption values for tea catechins (with detailed discussion reported in the dedicated section of this review). Some nations have covered this regulatory gap with national rules, as in the case of Italy. Indeed, the Italian Ministry of Health has allowed the use of EGCG from green tea as a bioactive ingredient in food supplements, with a maximum daily intake limit equal to 300 mg, with use discouraged during pregnancy (Ministero della Salute. ALTRI NUTRIENTI E ALTRE SOSTANZE AD EFFETTO NUTRITIVO O FISIOLOGICO, publication date: 6th August 2009).

As happens for food supplements, even in food, the use of extracted tea catechins is not allowed. In the United States, tea extract may be used as natural flavoring agent with antioxidative properties in foods characterized by high amounts of fat. According to the United States Code of Federal Regulations (21 CFR 101.22, and 21 CFR 182.10), the term "natural flavoring" means the *essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional*. In the European Union, the definition of "natural flavoring" substances, as stated in Council Directive 88/388/EEC, is: *a flavoring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption . . . Natural flavoring substances correspond to substances that are naturally present and have been identified in nature.* This definition is applied to the preparation of all foods, excluding those that require specific additional regulations. Some tea extracts are also considered to be traditional foods in Europe; however, the market classification of tea extracts depends on the intended use as well as the product profile. For example, if the

primary function relates to its antioxidant properties, the tea extract is regulated as an antioxidant agent and must be specifically approved.

18.7 Safety: Toxicity and Side Effects

The interest in tea catechins shown by the scientific community concerns both aspects related to their beneficial activity on human health and aspects related to their safety. The need for the evaluation of the safety of tea and tea catechins derives from case reports of adverse effects related to the consumption of green tea extracts or food supplements, and, in rarer cases, tea beverages. In 2009, in response to a request from the European Commission, EFSA published a safety assessment on green tea, focusing on dried extracts and traditional infusions consumed as foods, including beverages and food supplements in Europe. In 2018, the EFSA published a scientific opinion on the safety evaluation of green tea catechins (EFSA 2018). However, there are many gaps in the evaluation of the safety of tea catechins. These are due to various critical issues reflected in the collection and processing of data, such as the heterogeneity of the chemical composition of tea products or extracts, or of tea food supplements, tested in both toxicological and human intervention studies, and the lack of chemical characterization of these products, as well as a limited understanding of the mode of action or adverse outcome pathway for the observed toxicity.

The registered adverse effects in animal toxicological studies are hepatotoxicity, gastrointestinal toxicity, reduced weight gain, epicardium inflammation and myocardial necrosis, pancreatic necrosis, renal proximal tubular necrosis, nasal and olfactory toxicity, and thyroid dysfunction. The severity of injury is strictly related to the exposure (dose levels), the chemical composition of tea beverages or extracts, route of administration, and feeding state (fasting or bolus). In human intervention studies, gastrointestinal toxicity, hepatotoxicity, reduced body weight or weight gain, nasal-olfactory toxicity, and cardiotoxicity have been observed. This evidence is derived from a 2018 systematic review, which takes into account 104 peer-reviewed human clinical studies. The authors showed that only liver toxicity and gastrointestinal toxicity have a high relevance in humans, albeit with a very low incidence. While gastrointestinal toxicity was observed at lower dose levels than hepatotoxicity in most animal studies and all human studies, it was not considered to be a critical effect, because it is a local effect which could be readily prevented through consumption of tea products with or after a meal. Moreover, the authors reveal that tea catechins are not genotoxic or carcinogenic based on results from carcinogenicity and genotoxicity assays in experimental animals, corroborated by the lack of documented evidence from human epidemiological studies reporting any association between tea consumption and increased cancer risk (Hu et al. 2018).

In the 2018 EFSA opinion on the safety of tea catechins, the Panel considered that tea catechins cause a dose-dependent hepatotoxicity and that the consumption of tea catechins for 4 months or longer at a dose of about $>$800 mg EGCG/die is associated with an increase in the serum concentration of hepatic enzymes alanine

and aspartate aminotransferases, which are recognized indicators of liver injury (EFSA 2018).

In agreement with the opinion of EFSA, the United States National Institutes of Health stated that: "*Green tea extract and, more rarely, ingestion of large amounts of green tea have been implicated in cases of clinically apparent acute liver injury, including instances of acute liver failure and either need for urgent liver transplantation or death.*"

From the evidence gathered in the literature, it seems that among the different tea-based products taken into consideration, solid food supplements were found to be the most subject to adverse reactions. This finding is further confirmed by the PlantLIBRA study, a European project which studied the adverse effects of plant food supplements involving 2359 adults from six European countries (Finland, Germany, Italy, Romania, Spain, and United Kingdom). The self-reported adverse effects were registered in poison centers. The obtained results showed that the adverse effects deriving from the consumption of food supplements containing *Valeriana officinalis* and *Camellia sinensis* are the most diffused. The bias of this survey is that clinical evidence supporting the self-reported toxicity was not assessed.

The proposed mechanisms of liver toxicity reported in the literature are the loss of mitochondrial membrane potential, with the consequent increased production of reactive oxygen species and reduced glutathione contents. Another hypothesized mechanism is the pro-oxidative activity of tea catechins administered in high doses, which is mediated via formation of EGCG o-quinone, which reacts with protein thiols to form a compound with stress-generating properties. Moreover, hepatic toxicity may be induced by decreasing expression of heat shock proteins, which are synthetized in stress conditions, and antioxidant enzymes. Furthermore, genetic polymorphisms and pre-existing health conditions or concomitant medication use, such as nonsteroidal anti-inflammatory drugs, paracetamol or statins affecting mitochondrial membrane integrity, may predispose certain individuals to tea catechin-induced hepatotoxicity (EFSA 2018).

18.8 Marketed Products

Food supplementation can provide concentrated sources of nutrients or other substances that can have nutritional or physiological effects. The purpose is to supplement the normal diet. Food supplements are generally marketed in dose forms, e.g., as pills, tablets, capsules, or liquids in measured doses. Supplements can be used to correct nutritional deficiencies or to maintain an adequate intake of certain nutrients (http://www.efsa.europa.eu/en/topics/topic/supplements).

Antioxidants have been extensively used more recently to counter or overcome the effects of the excess ROS that are involved in several pathologies, including diabetes and obesity. Many over-the-counter dietary supplements that are used for "treatment" of obesity, neurodegenerative diseases, diabetes, and related metabolic disorders have insufficient medical information and support from scientific evidence.

Green tea has gained tremendous popularity as an all-round health "elixir," where it is described as burning fat and protecting against an array of illnesses, including cancers and CVD. With enticing names such as Green Tea Triple Fat Burner and Green Tea Slim, many people are drawn to such supplements with green tea for weight loss. Consumers spent about $140 million on these in 2015, according to data available from the Nutrition Business Journal (https://www.consumerreports.org/dieting-weight-loss/truth-about-green-tea-for-weight-loss). Indeed, the year 2019 is expected to remain strong for market growth of green tea extracts, primarily due to widening applications and strengthening buyer power. New growth opportunities will emerge across the green tea extract market value chain, with both suppliers and distributors focusing on adapting to the shifting consumer preferences. On the other hand, more intense competition and the demand for high-quality products at low cost will act as challenges for market growth to 2025 (https://www.marketresearch.com/OG-Analysis-v3922/Future-Global-Green-Tea-Extract-12199295/). On the eBay platform (https://www.ebay.com) at the time of writing, there were 335 products available from a search for green tea extracts under "dietary supplements" and 28 products that were said to contain tea catechins. Similar results were found on amazon.com (https://www.amazon.com/). Under "health and household," "vitamins and dietary supplements," "nutritional supplements" (295 products for this alone), "antioxidants," and "green tea extract," there were 163 products available.

These nutritional supplements that are said to contain green tea extracts can be found in the form of capsules, tablets, powders, or liquids. Based on the product information, these green tea extracts indicate the following claims: help to boost metabolism when combined with a reduced calorie diet and exercise plan (https://www.amazon.com/); a plant-based supplement that contains several antioxidants (and most importantly, polyphenols and EGCG) that have been shown to naturally increase resistance to diseases and degeneration; and more importantly for obese people, to support weight-loss and weight-maintenance goals. This last represents a staple for anyone looking to decrease their body fat. Some products contain more health claims, such as: Advanced Green Tea Formula, Zenwise Health Green Tea Extract (https://www.amazon.com/Green-Extract-Supplement-EGCG-Vitamin/dp/B00RH5I8U0), which claims to contain a blend of green tea extract (with EGCG and polyphenols) and vitamin C, to support weight loss, provide energy, and stimulate immune responses, cognition, and heart health for men and women; Metabolism Booster for Weight Loss, which claims that when combined with a healthy diet, the green tea extract (with EGCG catechin) is an effective fat burner that promotes slimming by giving the body metabolic function a natural boost; Antioxidant Immune System Support, which claims that with its 98% polyphenols, the green tea extract (*Camellia sinensis*) works with the vitamin C in this formula for a powerful source of antioxidants to bolster immune function to help you feel your best; No Caffeine for Jitter-Free Energy, which includes 725 mg green tea extract per serving and claims that this nature-inspired decaffeinated formula provides a pure and gentle boost of energy to get you through your day without any jitters or caffeine crashes; and Supports Brain & Heart Health, which as a daily green tea extract claims that this supplement promotes healthy cardiovascular function.

Although numerous in vitro and in vivo studies and clinical studies that have been reported here show the various beneficial effects described above, overall the data remain conflicting. More studies to validate their efficacy and safety are warranted, and large, well-designed, double-blind, placebo-controlled clinical studies need to be carried out before use of such preparations can be recommended for treatment and/or prevention of neurodegenerative diseases, diabetes, and other diseases. Some reports have also identified an association between hepatotoxicity and health supplements that contain green tea extract (Bonkovsky 2016; Garcia-Cortes et al. 2016; and references therein).

18.9 Patents

The various patents around these green tea extracts generally relate to the extraction methods used, along with the encapsulation of the tea catechins and the associated health claims. These patents also generally discuss the evidence found in the literature for the pharmacological activities of green tea. Indeed, as health-promoting products and disease-preventing applications, these green tea extracts, or compounds isolated from green tea extracts, are the subject of many patents. Using the Google Patents Searching Browser, more than 12,300 hits can be obtained for tea catechins (priority, 2002, granted, WO) (https://patents.google.com/). Here, we will restrict ourselves to only a few of what we see as the more important ones.

A US patent for methods and compositions for improving bioavailability of EGCG through administration of a nanoparticle complex to treat neurological diseases such as Alzheimer's disease or HIV-associated dementia was granted in December 2014 (US8906414B1). This invention was made with government support under Grant No. 5K08 MH082642-02 awarded by the National Institute of Mental Health (NIMH); Grant No. R43AT004871 awarded by the National Center for Complementary and Alternative Medicine (NCCAM); and Grant No. R21AG031037 awarded by the National Institutes on Aging. This patent is owned by Natura Therapeutics Inc., University of South Florida. The government has certain rights in this invention.

More recently, in December 2015, a patent application was filed with the US patent office for inhibition of neurodegenerative diseases using a "Grape seed extract, green tea extract and probiotic bacteria" (US20160106789A1), where the green tea extract is primarily EGCG. George Robertson filed a patent application (April 2015; WO2015154192A1) for use of a composition that comprises a flavan-3-ol, a flavonoid and a fatty acid in the treatment of oxidative injuries due to mitochondrial dysfunction. The disorders to be treated are indicated as neurodegenerative disorders, which include Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, and multiple sclerosis; neurological damage due to stroke; and ototoxicity arising from chemotherapy with cisplatin. EGCG is one of the possible flavan-3-ols present in the composition here.

Another patent defines "Agents for downregulation of the activity and/or amount of Bcl-xl and/or Bcl-w" (April 2013; WO2014174511A1; filed by Krizhanovsky V,

Pilpel N, Yosef R). This method comprises a therapeutically effective amount of an agent from a green tea extract that can downregulate an activity and/or an amount of Bcl-x L and/or Bcl-w and/or p21, with the proviso that the inflammatory disease is not cancer. In another patent (US6410061B1; filed by Morré DM, Morré JD, Purdue Research Foundation) defined for "Tea catechins as cancer specific proliferation inhibitors," the method and compositions for treating cancers or solid tumors comprise administration of a therapeutically effective amount of catechins, a group of polyphenols found in green tea, to a mammal in need of such therapy. These compositions of the catechins include, but are not limited to, epicatechin, ECG, EGC, and EGCG. This invention also covers the various modes of administration of the therapeutic compounds. Another patent (2014; US8906414B1; filed by Shytle RD, Tan J, Smith A, Giunta B, Sanberg CD) describes the compositions and methods of increasing the bioavailability of catechins by their encapsulation within nanoparticles, along with the compositions of the nanoparticle complex that are formed between the catechin and the nanoparticle. Each of these compositions was shown to increase the bioavailability of EGCG. This is defined as useful for treating diseases such as Alzheimer's disease and HIV-associated dementia.

Then there is "Topical liposome compositions containing phenolic anti-inflammatory agents and their methods of preparation" (June 2014; WO2014085914A1; filed by Simmons DL), where liposomal topical compositions containing phenolic anti-inflammatory agents, and methods for making thereof, are presented. These compositions are generally used to help to prevent solar radiation-induced skin damage and to help to prevent and/or treat inflammatory dry skin conditions caused by eczema and contact dermatitis. In the patent "Composition and method for improving cognitive function and brain bioavailability of ginseng and ginsenosides and treating neurodegenerative disease and neurological disorders" (February 2017; WO2018148821A1, filed by Kay DG & Maclellan A), the invention is directed to methods and compositions for improving cognition or rejuvenation of the brain of an individual using an active combination of at least one phospholipid and at least one substance selected from a group consisting of ginseng, green tea, catechin, ginsenoside, essential fatty acids, and combinations thereof. (https://patents.google.com/).

18.10 Perspectives

Tea obtained from the leaves of *C. sinensis* is one of the most popular beverages around the world. Several in vitro and in vivo studies have suggested that tea catechins can provide beneficial effects, which can include antioxidant, anti-inflammatory, antiviral, anticancer, antidiabetic, and anti-obesity activities, as well as activities against CVD and neurodegenerative diseases, among many other claims. However, the maximal concentrations of the catechins detected in blood in human subjects or animals after oral ingestion are in submicromolar or low micromolar levels, which indicate the small amounts that are absorbed and pass into the blood. These appreciably lower in vivo quantities compared to the effective concentrations

determined in in vitro studies suggest the poor stability of these compounds in the gastrointestinal tract.

The bioavailability of tea catechins has been extensively studied, and many so-called delivery systems have been proposed to improve their stability and promote their membrane permeability across the intestine. However, there are large differences in the conclusions from studies that have investigated the association between tea consumption and health benefits. In this respect, the solubility, structure, bioavailability, permeability, shelf life, safety aspects, and exact mechanisms responsible for the actions of tea catechins should be additionally examined in well-controlled human trials. Long-term dietary intervention trials in large populations with detailed information on lifestyle, dietary habits, and health status of participants have been proposed.

Many over-the-counter dietary supplements have insufficient medical information and support from scientific evidence if they are to be used for the treatment of diseases and metabolic disorders. Although numerous in vitro and in vivo studies, and also clinical studies, have reported beneficial effects of these tea catechins, the results have been, and remain, conflicting. More research is needed to validate their efficacy and safety and large, well-designed double-blind, placebo-controlled clinical studies need to be carried out before the use of such preparations can be recommended for treatment and/or prevention of health disorders.

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Theaflavins, Thearubigins, and Theasinensins

19

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Contents

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Abstract

Tea, except water, is the most widely consumed beverage across the world. It contains many active substances, among which polymeric polyphenols (theaflavins, thearubigins, and theasinensins) are dominant in fermented teas. Theaflavins and thearubigins are the major polyphenolic compounds in black tea, whereas theasinensins are the most characteristic for oolong tea. All of these classes of polyphenols have complicated structure and complex route of synthesis, in which polyphenol oxidase is a crucial enzyme. The aim of the present chapter is to provide a summary on the current knowledge on theaflavins, thearubigins, and theasinensins associated with chemical structure, molecular action pathways, and health benefits based on the scientific evidences available on the literature.

Keywords

Black tea · Catechins · Theaflavins · Thearubigins · Theasinensins

19.1 Introduction

Tea is a very popular drink in the world, and after water, it is the most frequently consumed beverage. The annual production of tea is estimated to around 2.9 billion tons. Every day almost 70% of the global population drinks 18–20 billion cups of tea. *Camellia sinensis* (L.) Kuntze belongs to the family Theaceae and is cultivated in over 45 countries from all continents, except North America and Antarctica. Subtropical and tropical climate with high precipitation and little acidic soil are the most preferable conditions for tea cultivation (Barua 2008). Young leaves, immature buds, and delicate stalks of tea bush are processed to produce tea as a food product. Taking into account the production process, tea can be divided into several types, of which green and black teas are the most popular ones. In the scientific literature, different classifications can be found; however recently Yi and co-workers (2015), based on the production process, divided tea into seven sub-types, namely, black, green, white, yellow, oolong, aged Pu-erh, and ripened Pu-erh. Green and white teas are non-fermented products, while the latter one is considered high-quality tea produced from young leaves and buds harvested before their maturation, mostly in spring. The tea leaf contains over 4000 active constituents. Table 1 summarizes the composition of tea according to the leaf and to the tea infusion.

Polyphenols are the main and most widely distributed secondary metabolites present in plants. They can be divided into several subclasses of which flavonoids are the most complicated and widespread in the plant kingdom (Fig. 1).

The basic structure of flavonoids, so-called flavan skeleton, consists of two aromatic rings linked by a three-carbon atom heterocyclic ring. All flavonoids contain OH groups in the positions 5- and 7- in the A ring and in the position 3- in the B ring; however the differences in location and character of other OH groups and the presence or absence of a double bond between carbon atoms 2 and 3 in the C

Table 1 The composition of tea

Composition of fresh, unprocessed leaves (dry weight)	Composition of water infusion, prepared from fresh unprocessed tea leaves (dry weight)
Polyphenols 36%	Polyphenols: Catechins 30% Simple polyphenols 2% Flavonols 2% Other polyphenols 6%
Carbohydrates 25%	Carbohydrates 11%
Proteins 15%	Proteins 6%
Theanine 4%	Theanine 3%
Free amino acids 1–5.5%	Free amino acids 3%
Fats 2%	Fats 3%
Alkaloids: Caffeine 3.5% Theobromine 0.15–0,2% Theophylline 0.02–0,04%	Alkaloids: Caffeine 3% Other methylxanthines <1%
Organic acids 1.5%	Organic acids 2%
Ash (inorganic compounds) 5% Lignin 6.5% Chlorophyll and other pigments 0.5% Carotenoids and volatiles <0.1%	Potassium 5% Other inorganic compounds 5% Volatiles (traces)

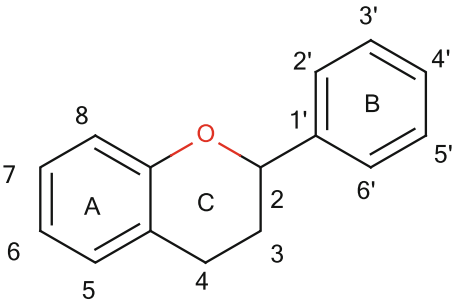


Fig. 1 Flavan skeleton – a basic structure of flavonoids

ring are the reasons for the division of this group of compounds into several subclasses: flavanones, flavonols, flavones, isoflavones, anthocyanidins, and flavan-3-ols. The latter one, also called catechins, are the main active constituents of tea. A characteristic feature of these substances is the presence of the -OH group in position 3 in the C ring (Fig. 2).

The major catechins present in the tea leaves, alongside with their abbreviations used in the literature, are (−)-gallocatechin (GC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epicatechin (EC), (−)-gallocatechin gallate (GCG), and (−)-epigallocatechin-3-gallate (EGCG). Their structures were presented below (Fig. 3).

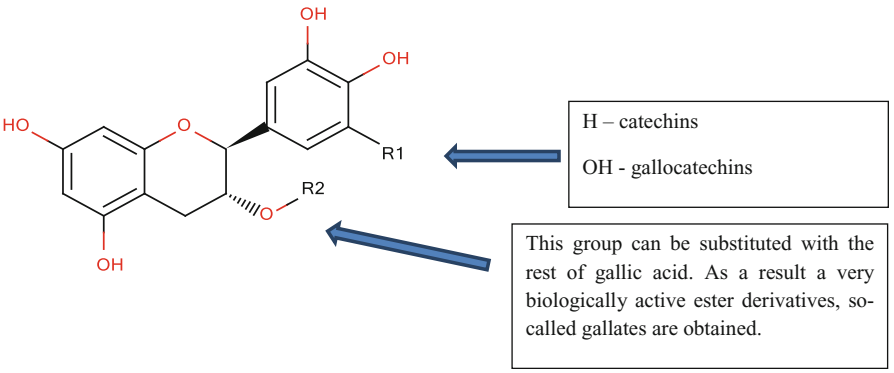


Fig. 2 The structure of flavan-3-ols

During the fermentation, which is the main process in the production of black tea, over 75% of catechins are transformed into complicated polymers, such as theaflavins (TFs), thearubigins (TRs), and theasinensins (TSs).

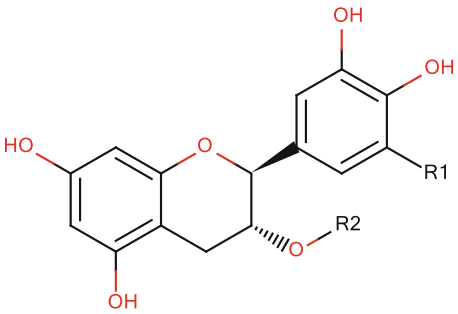
19.2 Theaflavins

19.2.1 Biosynthesis and Structure

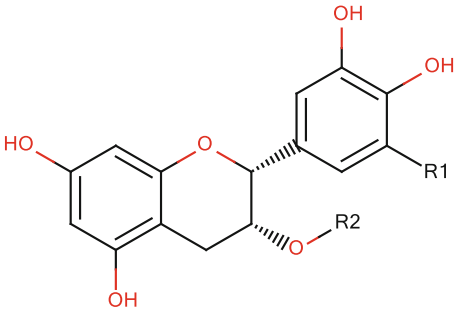
The composition of black tea was presented in Table 2.

Theaflavins and thearubigins are the major polyphenolic compounds in black tea. The first accounts for 2–6% of solids in the dry matter of the brew of the black tea. TFs are synthesized during the dimerization process of catechins. So far more than 25 theaflavins were found in the black tea. The main element of their structure is the seven-membered benzotropolone ring, which is formed due to the oxidation of the epigallocatechin ring B or epigallocatechin gallate, followed by decarboxylation and simultaneous fusion with the epicatechin ring B or epicatechin gallate (Beecher 2003). The most important theaflavins found in black tea are theaflavin (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3′-gallate (TF_2B), and theaflavin-3,3′-digallate (TF_3). In literature some other signatures of TFs can be found, namely, TF_1 as TF, TF_2A as TF_1, and TF_2B as TF_2. Theaflavin-3,3′-digallate is always marked as TF_3 (He 2017). In addition to theaflavins in tea infusions, the presence of other compounds that are derivatives of benzotropolone, among others theaflagalline or theaflavin acids, was proofed. The structures of four main TFs alongside with the basic monomers were illustrated in Fig. 4.

The relative proportion in which particular theaflavins are present in black tea was reported to be as follows: theaflavin (18%), theaflavin-3-gallate (18%), theaflavin-3′-gallate (20%), theaflavin-3,3′-digallate (40%), and minor derivatives such as theaflavic acids.



	R_1	R_2
(+)-catechin	H	H
(−)-gallocatechin	OH	H
(+)-catechin gallate	H	Gallic acid
(−)-gallocatechin gallate	OH	Gallic acid



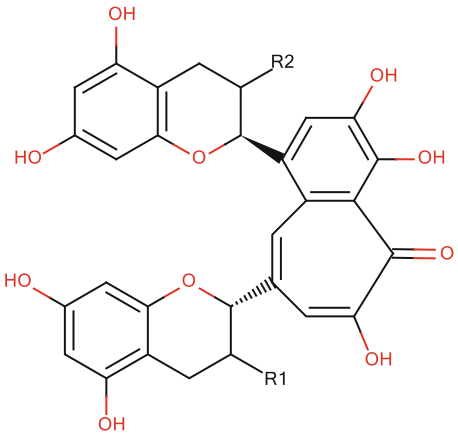
	R_1	**R_2**
(-)-epicatechin	H	H
(−)-epigallocatechin	OH	H
(−)-epicatechin gallate	H	Gallic acid
(−)-epigallocatechin-3-gallate	OH	Gallic acid

Fig. 3 Structures of the tea catechins

The reactions of oxidation and polymerization of catechins probably occur with the participation of two enzymes: polyphenol oxidase and peroxidase. It is believed that mainly polyphenol oxidase is responsible for the oxidation of flavanols to

Table 2 The composition of black tea

Compounds	Content (% of dry matter)
Polyphenols	
Flavonoids (mainly catechins, but also flavonols, flavones, phenolic acids, and proanthocyanidins)	5
Thearubigins and theaflavins	25
Proteins	15
Amino acids	4
Carbohydrates (including fiber)	33
Fats	2.5–7.0
Alkaloids (mainly caffeine)	4
Pigments (mainly chlorophyll and carotenoids)	2
Volatiles	0.1
Minerals	5



	R_1	R_2	Monomers
Theaflavin (TF_1)	OH	OH	EC+EGC
Theaflavin-3-gallate (TF_2A)	Gallic acid	OH	EC+EGCG
Theaflavin-3'-gallate (TF_2B)	OH	Gallic acid	EGC+ECG
Theaflavin-3,3'-digallate (TF_3)	Gallic acid	Gallic acid	ECG+EGCG

Fig. 4 Structures of tea theaflavins (Lambert and Yang 2003)

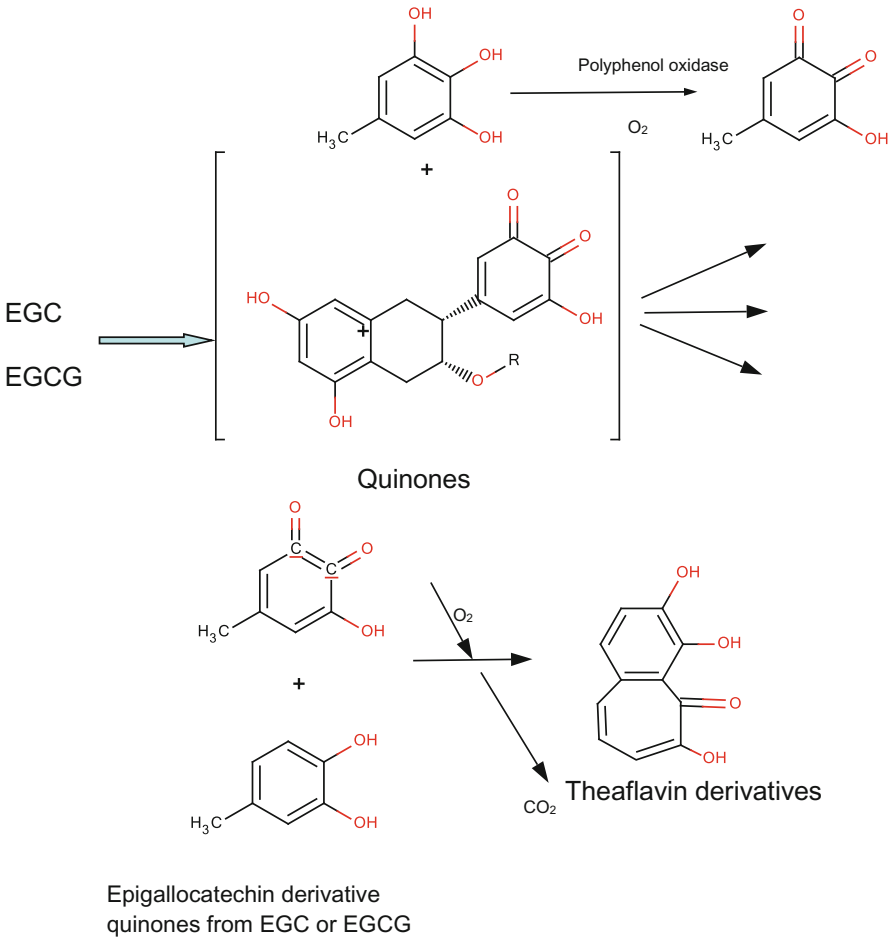


Fig. 5 The probable mechanism of synthesis of theaflavins during the fermentation process (Haslam 2003)

theaflavins and thearubigins (Haslam 2003). Peroxidase activity, which catalyzes the oxidation reaction of phenols to quinones with the participation of hydrogen peroxide, has been shown to be five times higher in fresh tea leaves compared to the oxidase, and its activity increases during the production process. However, its role in the synthesis of complicated polymeric polyphenols of black tea has still not been fully explained (Mahanta et al. 1993) (Fig. 5).

19.2.2 Organoleptic Properties

Theaflavins are orange or orange-red pigments, which determine the specific color and taste of black tea infusion. TFs were called a "golden molecules," because of

their wide biological properties. Very often they are associated with the grade and quality of black tea, the most consumed tea in the world. Although both theaflavins and thearubigins are responsible for the specific taste and aroma of black tea, the first one are considered as the major group of compounds used to assess the market value, clonal variations, and seasonal quality variations of black tea (Yao et al. 2006). Theaflavins have astringent properties and give specific mouth-coating and long-lasting oral sensation at the back of the throat. However their astringent properties are much lower, compared to catechins, and account for about 0.1% of the total astringency of all tea constituents (He 2017).

19.2.3 Absorption and Metabolism of Theaflavins

Bioavailability of theaflavins is very poor. Based on the plasma and urine concentrations, Mulder and co-workers established the intake rate at 0.0006%, and Pereira-Caro and co-investigators obtained even lower value of 0.000001% after oral ingestion of a mixture of theaflavins, which was an equivalent of ~10 cups of black tea (Mulder et al. 2001; Pereira-Caro et al. 2017). Ingested TFs pass through the gastrointestinal tract unchanged to the colon, where they are subjected to the action of the resident microbiota. As a result low molecular weight degradation products are formed, mainly hydroxybenzene derivatives, particularly pyrogallol-1-sulfate, pyrogallol-2-sulfate and 3-(4′-hydroxyphenyl)propionic acid. Probably theaflavins are catabolized in the two pathways – first by colon microbiota and second by mammalian cell enzymes during phase II metabolism. Because of a very low TFs bioavailability, the beneficial role associated with the consumption of black tea is linked to their circulating metabolized products, which are mainly of microbial origin (Pereira-Caro et al. 2017).

19.2.4 Bioactivity of Theaflavins

In recent years many beneficial effects of TFs were revealed including in vitro and in vivo studies. Below some of the most important biological activities were described (examples were limited to animals and human studies).

19.2.4.1 Antioxidant Activity

It is already well-known and was confirmed in numerous studies that tea infusions possess strong antioxidant activity. Black tea infusions are now considered as a major source of dietary polyphenols for a general population. For many years this product was thought to be a weaker dietary antioxidant agent, compared to green tea, which seemed obvious, because over 75% of simple catechins are decomposed during fermentation process. However, recent findings revealed that the new compounds (e.g., theaflavins), which arise, are also characterized by strong radical scavenging activity. The ability to quench DDPH (2, 2-diphenyl-1-picrylhydrazyl) radical by theaflavins can be expressed as follows: $TF_3 > TF_2B = TF_2A > EGCG > TF_1$

(He 2017). This comparison shows that three major theaflavins are stronger antioxidants than EGCG, which is considered one of the most active antiradical molecules present in food. The randomized, double-blind, crossover study performed by Arent and co-workers on humans subjected to acute anaerobic interval training revealed beneficial effects of TFs intake. The study showed that the consumption of black tea enriched in theaflavins led to better recovery and reduced the oxidative stress. Moreover it reduced muscle pain caused by long anaerobic periods (Arent et al. 2010).

19.2.4.2 Antimutagenic Activity

The study performed on bone marrow cells of mice showed significant decrease in chromosomal aberrations and sister chromatin exchange caused by benzo[a]pyrene, after oral administration of black tea extract, which was equal to the consumption of five cups per day. This activity was mostly due strong antioxidant activity, which inactivated direct carcinogenesis (desmutagenicity) outside the cell. Other proposed mechanism linked this activity with the induction of P450 cytochrome enzymes, which results in the activation of intracellular detoxification of carcinogens. Moreover induction of DNA repair, inactivation of reactive forms of mutagens and carcinogenesis, as well as inhibition of promutagen activation, promotion, invasion, and metastasis of tumor cells are also mechanisms which underline the antimutagenic activity of theaflavins (Halder et al. 2005).

Wang and co-workers proved protective effects of theaflavins on cadmium-induced (0.4 mg/kg body weight, s.c., once a day) testicular toxicity in male Sprague-Dawley rats. The animals received 50, 100, and 200 mg/kg of TFs per body weight, orally, once a day during 5 weeks. The study revealed dose-dependent reduction of cell damage in testis and DNA damage, increased serum testosterone levels, and improved sperm characteristics. The authors also observed other beneficial effects of theaflavins administration, e.g., decrease in the production of MDA caused by Cd; reduced concentration of this metal in the liver, testis, and blood; and increased excretion of cadmium with feces and urine (Wang et al. 2012).

19.2.4.3 Influence on Blood Lipids

Theaflavins were proved to inhibit the lipase and amylase and suppress the expressions of lipopolysaccharide-induced intercellular adhesion molecule and vascular cell adhesion molecule by blocking the activation of NF-κB and JNK in the epithelial cells of the intestine. Additionally TFs suppress lipogenesis by inhibiting fatty acid synthase, through downregulation of epidermal growth factor and receptor/PI3K/Akt/Sp-1 signal transduction pathway. As a result of all of these mechanisms, significant hypolipidemic effect was observed.

TFs were also shown to have strong effects toward the prevention of obesity by inhibition of hepatic lipid storage, reduction of lipid accumulation, and production of fatty acids and stimulation of their oxidation. After oral administration of TFs, increased energy expenditure and expression of metabolic genes were proved. Theaflavins are also considered to improve liver steatosis, oxidative stress, and inflammation and as a result decrease hepatocyte apoptosis (He 2017).

19.2.4.4 Anti-inflammatory Activity

Gosslau and co-workers proved TF_2B to have strong anti-inflammatory effect by the suppression of the 12-O-tetradecanoylphorbol-13-acetate-induced COX-2 gene expression and downregulation of TNF-α, inducible nitric oxide synthase (iNOS), ICAM-1, and nuclear factor κB (NF-κB). They have also reported that TF_2B significantly reduced ear edema in mouse, by producing similar pattern of gene downregulation to that observed in the cell model (Gosslau et al. 2011). TF_3 was shown to have protective effect in colitis. Theaflavin derivatives were also proved to decrease the expression of the inflammatory cytokine IL-6, one of the most important mediators of inflammation (He 2017).

19.2.4.5 Antimicrobial Activity

Theaflavin-3,3′-digallate (TF^3) during the epidemiological studies was shown to be strong inhibitor of 3C-like protease, related to severe acute respiratory syndrome (SARS). TFs were proved to have strong inhibitory effects against influenza and HIV viruses, by blocking the initial steps of infection through attenuation of neuraminidase and hemagglutinin activity (He 2017).

Other studies, performed using six clinical isolates of herpes simplex virus type 1 (HSV-1) and two clinical isolates of HSV-2, have shown that the combination of TF_3 and lactic acid significantly reduced the infection caused by these viruses in the pH spectrum of 4.0–5.7, which mimic the conditions found in the female reproductive tract (Isaacs and Xu 2013).

Betts and co-workers revealed strong antibacterial effect of various concentrations of theaflavins toward eight clinical isolates of *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*, important nosocomial pathogens, resistant to multiple antibiotics. They also proved significant synergism of action with epicatechin; however the mechanism of such activities was not elucidated (Betts et al. 2011).

19.2.4.6 Anticancer Activity

TFs were shown to have strong anticancer activity in numerous studies performed on human cancer cell lines, by blocking extracellular signal transmission and cell proliferation and improving cell shrinkage, membrane blebbing, and mitochondrial clustering. As a result of these mechanisms, the induction of cancer cells apoptosis was observed (He 2017).

Oral administration of theaflavin-enriched black tea extracts (40% of TFs) to Sprague-Dawley rats with dimethylnitrosamine-induced liver fibrosis reduced necrosis, bile duct proliferation, inflammation, and elevated levels of serum GOT (glutamate oxaloacetate transaminase) and GPT (glutamic pyruvic transaminase). Studied extract was efficient at the dose of 50 mg/kg as well as 100 mg/kg per day. Hepatic fibrosis is a high-risk factor of developing hepatocellular carcinoma (HCC), which is the fifth most common cancer in men and the seventh in women globally (Weerawatanakorn et al. 2015a). Thus drinking black tea may have beneficial role in the prevention of HCC.

Tea polyphenols (including theaflavins) were observed to suppress the activation of aryl hydrocarbon receptor (AhR) induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin

(TCDD) ex vivo in rats. Conjuncted and intact forms of catechins and theaflavins were present in the plasma and liver of animals. These results confirmed that polyphenols from tea can be incorporated in the liver and through the AhR activation pathway may inhibit the activity of cytochrome P450 1A1 enzyme. Tea phenolics are also a promise chemopreventive agent in human prostate cancer. The relative bioavailability of theaflavins in the prostate tissue was 70% higher compared to EGCG. It was proved that tea polyphenols (catechins and theaflavins) are bioavailable in prostate, where they can act as anticancer agents (He 2017).

Lee et al. reported that theaflavin gallates are hydrolyzed by salivary esterases, which were proofed in either in vitro or in vivo studies, and high concentrations of theaflavins were observed in saliva in the first hour after oral administration of black tea brew. This might reflect that black tea infusions may play beneficial role in the prevention of oral cancer and dental carries (Lee et al. 2004).

19.2.5 Safety: Toxicity and Side Effects

Theaflavins should be considered as a safe and nontoxic natural product. In the literature there is no information on toxicity of TFs. The only information which might be found is that TF_2A and TF_2B could act as a prooxidants and increase oxidative stress. However it was observed in relation to cancer cells, which must be considered a beneficial activity (Babich et al. 2008).

19.2.6 Marketed Product

Theaflavins are present on the market as dietary supplements containing black tea extracts. *Theaflavin Standardized Extract*® produced by LifeExtension® contains 350 mg of black tea extract, which is standardized to 25% of theaflavins per one capsule. The product is protected by US patent Nos. 6,811,799 and 6,602,527 and can be bought on the US market. The producer does not inform what are the proportions of particular theaflavins in the extract.

Theaflavin® produced by Seeds® is also a dietary supplement which contains black tea extract, together with green tea extract and vitamin E. However, the producer does not provide any information on the composition of this extract, except that it was produced from premium black tea. This product is also offered in the US market.

19.2.7 Perspectives

Taking into account lack of toxicity and side effects, numerous beneficial effects to the organism, which was presented above, and high daily intake with very popular black tea infusions, theaflavins can be potentially used in functional foods and nutraceuticals in the prevention of several lifestyle diseases, e.g., obesity, cancers,

or diabetes. Other potential applications include liver diseases, dental carries, and reduction of toxicity caused by heavy metals (e.g., cadmium). Theaflavins are consumed every day by a large population of the world with black tea, so probably they are still acting, although we do not note or measure the effects of their action. Except that, TFs can be used as isolated compounds or purified extracts from tea as dietary supplements.

19.3 Thearubigins

19.3.1 Biosynthesis and Structure

Thearubigins (TRs) are the next polymeric polyphenols present in black tea, which (together with theaflavins) are responsible for specific brown color and aroma of the infusions. In the past TRs were considered as the insoluble fraction of ethyl acetate extraction of the black tea (Yao et al. 2006). Their content in black tea is about 12–18%, and therefore they comprise the biggest part of all active constituents. TRs are synthetized during the same process as TFs, in which polyphenol oxidase and peroxidase are involved.

Thearubigins are compounds with a red-brown or dark brown color. Although the majority of catechins during fermentation are likely to be transformed into thearubigins, the structure and formation of these substances are still poorly understood. They are believed to be heterogeneous flavan-3-ols and their gallate derivates (Fig. 6). Some authors also suggest that theaflavins are involved in the formation of thearubigins (Haslam 2003). It seems that the pathway of TRs synthesis mainly involves EGC and EGCG and in the next steps quinone and then theaflavins and other benztropolones. Another route suggests participation of theasinensins.

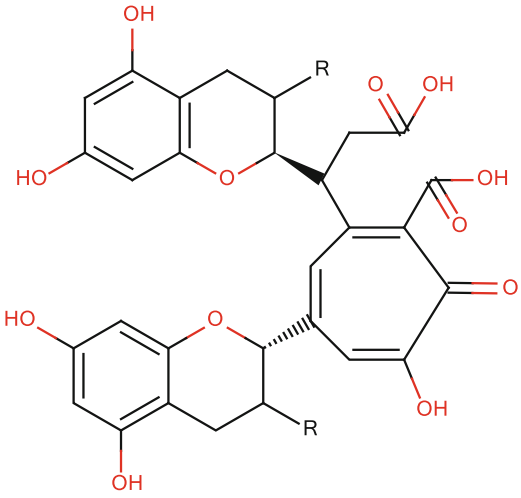


Fig. 6 The proposed structure of thearubigins (Khan and Mukhtar 2007)

The process of thearubigins synthesis resembles "the browning" – very often observed in nature, during which the plant tissues are damaged and oxidized, based on the quinone tanning, the Maillard reaction, or caramelization. TRs are a group of very closely structurally related polymeric compounds, which range from dimeric and trimeric to tetrameric and even greater structures and average molecular weight of ~700–2000 (Haslam 2003).

19.3.2 Organoleptic Properties

Some of the authors suggest that most of the catechins present in fresh tea leaves are transformed into thearubigins during the fermentation process and therefore they constitute the final group of compounds during the production of black tea. TRs are water-soluble, acidic compounds, primarily responsible for rust brown color of the infusion. They are characterized by ill-defined chromatographic behavior, and since many years, little progress has been made toward an understanding of their chemical nature. It is proposed that thearubigins are mainly responsible for the red-brown color of the tea infusion; however its specific taste and aroma are mostly due to the presence of theaflavins (Khan and Mukhtar 2007). Due to their very high water solubility, thearubigins account up to 30–60% of the solids in black tea infusion. TRs are very often associated with the richness of taste and therefore called a "body" of black tea (Yao et al. 2006).

19.3.3 Biological Activity

There are very few data on biological properties of thearubigins. Since their structure was not fully elucidated, there are no standards on which studies toward their activity may be conducted. Because there are very few information on that subject, below some studies on biological activities of TRs were presented, which can be found in the scientific literature.

Halder and co-workers proved significant antimutagenic effect of thearubigins. Fraction of TRs was extracted from black tea infusion by n-butanol liquid-liquid partition. Particular compounds were not separated and identified, but the total fraction was described as "thearubigins." For chromosome aberration assay (CA), the animals were fed by gavage and obtained three different concentrations of TRs (40, 80, and 160 mg/kg body weight). Immediately after the gavage, the animals were administrated benzo[a]pyrene (B[a]P) (100 mg/kg body weight). For the analysis of CA, the animals received colchicine, and 2 h later they were killed, and their bone marrow was expelled and subjected to the analysis. The study revealed significant reduction in CA in all the three different concentrations of thearubigins with B[a]P compared to the control group. The treatment with TRs significantly reduced both the chromatid and chromosome types of aberrations (Halder et al. 2005).

The same authors performed in vivo sister chromatid exchange (SCE) test in mice using TR fraction. All the steps of the treatment were the same as in CA analysis. Obtained results showed significant anticlastogenic effect of orally administrated thearubigins as measured by SCE against B[a]P – a strong mutagen. All three tested concentrations of TRs showed significant reduction toward the frequency of SCE (Halder et al. 2005).

TRs showed significant antimutagenic properties against B[a]P in the in vitro plate test using four different Salmonella strains (TA97a, TA98, TA100, and TA102). Obtained results showed significant antimutagenic activity of thearubigins.

The authors concluded that in the CA assay, TRs showed two times lower protective effects compared to TFs fraction, and in the SCE study, TFs revealed about three times more protective effects compared to TRs. Interestingly thearubigins showed different activity in two different test systems. Their activity was much higher in in vivo mammalian cells than in in vivo bacterial systems. Antimutagenic and anticlastogenic activity of thearubigins is the same as in the case of theaflavins, probably due to their antioxidant properties, which inactivate the extracellular activity of direct carcinogens. Thearubigins also increase the activity of cytochrome P450, which results in the enhancement of the intracellular detoxification of carcinogens.

Catterall and co-workers investigated antimutagenic effects of theafulvins (a fraction of thearubigins) in vivo using male Wistar albino rats, against several dietary carcinogens (heterocyclic amines, polycyclic aromatic hydrocarbons, and nitrosamines). The study showed concentration-dependent significant reduction of the mutagenicity of 2-amino-3-methyumidazo-[4,5-f]quinoline, 2-amino-l-methyl-6-phenylimidazo[4,5-fc]pyridine, benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, nitrosopyrrolidine, and nitrosopiperidine. However the mutagenicity of aflatoxin B1 was increased (Catteral et al. 1998).

The antioxidant activity of black tea is well-known and is due to the simple phenolics like catechins, as well as theaflavins and thearubigins. All of these groups of compounds, because of their phenolic nature, have strong antioxidant properties. However the knowledge and studies on this subject for particular polyphenols of black tea may be classified as follows: catechins>TFs> > TRs. The antioxidant activity of thearubigins is definitely the least well-known.

Study on the antioxidant activity of TRs rich fraction, performed using different in vitro and in vivo systems (e.g., DPPH, ABTS, FRAP, or hydrogen peroxide assays and protection of different biomolecules against oxidative stress in rats), revealed similar activity of both thearubigin- and theaflavin-rich fractions. Once again it was proofed that strong antioxidant potential of black tea is the resultant of activity of all compounds of phenolic nature present in black tea leaves. Thus in some situations, black tea is described as a more potent antioxidant agent compared to green tea (e.g., stronger lipid peroxidation inhibitor) (Sinha and Ghaskadbi 2013).

Imran and co-workers studied the influence of theaflavins and thearubigins on antioxidant status and lipid peroxidation in rats. They have proved that TFs exhibited higher antioxidant activity. Animals receiving a diet rich in both TFs and TRs were characterized by significant reduction in lipid profile, glucose content, and renal

function. Moreover this study revealed that animals receiving TFs were characterized by lower values in the TBARS test (thiobarbituric acid reactive substances), which is one of the most widely used assays for measuring lipid peroxidation end product – malondialdehyde and lipid profile, compared to thearubigins or a mixture of TFs and TRs (1:1). On the other hand, theaflavins+thearubigins diets caused the highest glucose, urea, and creatinine decrease and maximum level of insulin and antioxidant parameters compared to diets containing theaflavins or thearubigins alone (Imran et al. 2018).

The antioxidant activity of particular constituents of black tea can be characterized as follows: TF_3 > EGC > the mixture of TF_2A and TF_2B > thearubigins>TF_1. It should be also noted that studies regarding the antioxidant activity of particular black tea polyphenols are inconclusive, mainly because of different systems and parameters used. However many data suggest that the strongest active compound is TF3, followed by simple catechins. Thearubigins, although considered as strong antioxidant agents, are classified much less active compared to catechins or theaflavins.

19.3.4 Conclusion

Because of the limited data and only few scientific studies regarding thearubigins, it is not easy to predict a future perspective for this group of compounds. There are no information on toxicity of these compounds, but taking into consideration that they are a basic constituent of black tea, thus a significant ingredient of human daily data, their toxicity and side effects should be considered low.

Except dietary supplements containing total extracts from the black tea or isolated theaflavins, there are no products on the market with isolated thearubigins or fractions rich in these compounds. However majority of people consume TRs every day while drinking black tea infusions. Taking into account how popular is this beverage, thearubigins should be considered significant constituent of human diet, being simultaneously one of the most important non-nutrients.

19.4 Theasinensins

Black tea is definitely the most popular type of tea, which accounts for almost 78% of the global production. It is mainly consumed in the United States and Europe. Green tea is mostly preferred in Asian countries, and its market share is estimated to be ca. 20%. The third most popular type is oolong tea, consumed mainly in Taiwan, southern China, and many Eastern countries. Although its production is only 2% of the global market and thus it is far below the most popular types, namely, black and green, its popularity is still increasing. During the last two decades, its production in China has almost doubled, from 67.6×10^3 metric tons in 2000 to 254×10^3 metric tons in 2014 (Weerawatanakorn et al. 2015b).

Table 3 The composition of oolong tea beverage (Sajilata et al. 2008)

Compound	Content (mg/100 mL)
Catechin	1.65
Gallocatechin	6.65
Epigallocatechin	16.14
Epicatechin	5.08
Catechin gallate	0.6
Epicatechin gallate	5.73
Epigallocatechin gallate	25.73
Gallocatechin gallate	1.85
Gallic acid	2.19
Caffeine	23.51
Polymerized polyphenols	33.65
Total polyphenols	99.32

Oolong is a semi-fermented type of tea, and the degree of catechin oxidation is in a wide range of 10–80%, depending on the consumer requirements. Apart from the degree of fermentation, all three types of tea are also differentiated based on its amino acid content, mainly L-theanine but also alanine, leucine, serine, or glutamic acid. Oolong tea has specific taste and aroma, depending on several parameters like smell of volatile fragrance, umami (specific taste, hard to explain), and degree of astringency versus sweetness (Alcazar et al. 2007).

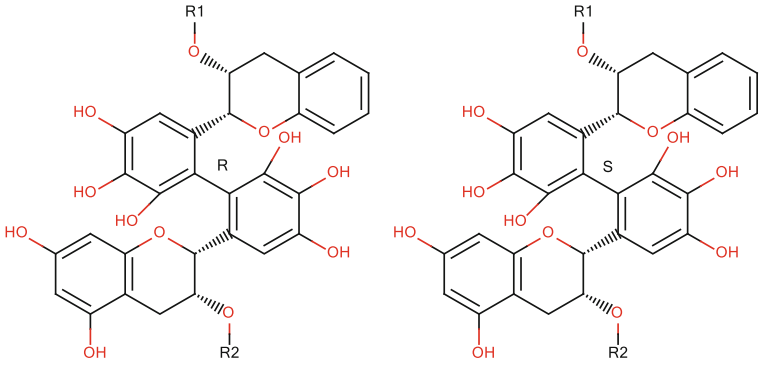
Major components of oolong tea can be divided into two groups: simple catechins and polymeric polyphenols. Since this type of tea is a semi-fermented product, the degree of catechin degradation changes, and thus the concentration of polymeric compounds differs. Sajilata and co-workers characterized the composition of oolong tea beverage as follows (Table 3).

Major secondary polyphenols in black tea are theaflavins and thearubigins, which were previously described. However oolong tea contains another class of polymeric polyphenols, known as theasinensins.

19.4.1 Biosynthesis and Structure

In 1958 Robert discovered a new group of compounds, known as bisflavanols A, B, and C, which were formed by linkage of two EGCG molecules. Later, in 1984, this group of polymeric oxidized flavan-3-ols was isolated from oolong tea and identified as theasinensins A, B, and C. In 1988 Nonaka and Hashimoto confirmed the presence of other compounds of this type, which were named using the letters D, E, F, and G (Weerawatanakorn et al. 2015b).

Theasinensins are considered as bioactive flavonoids of oolong tea. They are formed by the coupling of two catechins at their B rings through a C-C molecular bond. Its structure was presented in Fig. 7.



R stereoisomers		S stereoisomers	
Theasinensin A	R1=R2=Galloyl	Theasinensin D	R1=R2=Galloyl
Theasinensin B	R1=Galloyl R2=H	Theasinensin E	R1=R2=H
Theasinensin C	R1=R2=H		

Fig. 7 The structures of theasinensins (Weerawatanakorn et al. 2015b)

The structures of theasinensins A, B, and C contain the biphenyl bonds in the configuration R, whereas in D and E, it is R-biphenyl configuration (Fig. 7). Some authors suggest that theasinensins D and E are just stereoisomers of A and C. If we consider basic monomers which build particular TSs, it visible that theasinensins A and D are dimers of EGCG with an R- and an S-biphenyl bond and theasinensin B is the dimer formed by the conjunction of EGCG and EGC, whereas theasinensin C is a dimer of EGC (Tanaka et al. 2003; Matsuo et al. 2006).

Theasinensins can be found in fermented teas, namely, black and oolong. They are synthetized during the fermentation process, in which the two dominant catechins in fresh tea leaves – EGCG and EGC – are oxidized by polyphenol oxidase. As a result corresponding quinone derivative is produced, which undergoes stereoselective dimerization to produce theasinensin (Tanaka et al. 2003).

The pathways in which TSs are formed are very interesting. Hashimoto concluded that theasinensins are much more easily synthetized by polyphenol oxidase, compared to theaflavins (Hashimoto et al. 1992). The crucial point during the formation of TSs is the enzymatic oxidative coupling of pyrogallol rings of EGCG. This reaction could be also conducted using chemical agents. There are different pathways in which theasinensins and theaflavins are formed. The latter one are formed during the oxidative condensation reaction between catechol moiety of EC or ECG and a pyrogallol moiety of EGC and EGCG, which was described

previously. The pathway in which theasinensins are formed involves conjunction of pyrogallol-type B rings of EGCG and EGC. TFs are synthetized in the first step of black tea production, when leaves are crushed and kneaded. At this point TSs are not observed. They are formed when the leaves are heated in the temperature of 80 °C. One of the most important intermediates during the synthesis of TSs is unstable dehydrotheasinensins. They are formed from the oxidation of pyrogallol-type B ring catechins. During the final stage of black and oolong tea production (heating and drying process of the tea leaves), dehydrotheasinensins undergo redox dismutation to form corresponding theasinensins. It was elucidated that the transformation of dehydrotheasinensin A gives theasinensins A and D. Moreover it was implied that this process may be a nonenzymatic process, which spontaneously occurs during heating and drying of the tea leaves. Such a process may be also conducted in vitro in the nonenzymatic way (Weerawatanakorn et al. 2015b). Shii and co-workers obtained dehydrotheasinensin A by a nonenzymatic oxidation of EGCG by copper salts at the room temperature and pH 4–5. The efficacy of the reaction increased with the elevated temperature (Shii et al. 2011).

19.4.2 Biological Properties of Theasinensins

Although the scientific literature is rich in information on the biological properties of green and black teas, the data regarding oolong tea and its main active compounds – theasinensins – are still limited. Because majority of experiments evaluating biological activity of theasinensins are in vitro experiments, and oolong tea is the tea most equated with theasinensins, below in vivo studies on the activity of the pure compounds as well as its main source were presented.

19.4.2.1 Antioxidant Activity

It is obvious that, taking into account, that the main active ingredients of oolong tea are polyphenols, this product will be strong antioxidant agent – similar to green and black teas. In vivo study revealed that the consumption of oolong tea temporarily moderately increased the antioxidant capacity of human plasma. It was further observed that oolong tea reduced oxidative stress and DNA damage caused by free radicals (Higdon and Frei 2003). Study performed on athletes revealed that the consumption of oolong tea for 30 days significantly reduced the levels of malondialdehyde at rest and after exercise. The resting levels of SOD (superoxide dismutase) were also reduced. Moreover it was found that oolong tea decreased the level of lipid peroxidation and normalized the cholesterol profiles (Tsai et al. 2005). Theasinensin C, among all theasinensins isomers, was considered as the strongest antioxidant agent against lipid peroxidation (Weerawatanakorn et al. 2015b).

19.4.2.2 Anti-inflammation

Several studies demonstrated anti-inflammatory activity of oolong tea and its active constituents. It was shown that ethanol extract from oolong tea increased angiogenesis and adiponectin gene expression in epididymal fat, which was considered as

anti-inflammatory effect. Moreover this extract was proofed to have beneficial effects on metabolism and inflammation through pro-angiogenic activity during adipose tissue expansion (Weerawatanakorn et al. 2015b). On the molecular basis, it was shown that ethanol extract from oolong tea reduced the concentration of monocyte chemoattractant protein-1 (MCP-1) in serum, a small cytokine responsible for gathering monocytes, memory T cells, and dendritic cells to the site of injury (Tchernof and Despres 2013). Moreover it was elucidated that theasinensins A and D reduced LSP-induced COX-2 and PGE_2 synthesis, and this effect was stronger in the case of theasinensins B, C, and E. It was proposed that the galloyl moiety (present in the isomers A and D) plays an important role in anti-inflammatory activity of this group of compounds (Hou et al. 2010). It was also discovered that theasinensin A caused changes in 1382 genes responsible for chemokines, interleukins, and interferons production, thus strongly interfering in the immune defense system (Chen et al. 2011). Continuation of experiments elucidated new molecular pathways of the influence of theasinensins on immune defense components – theasinensin A significantly reduced the levels of inflammatory mediators like inducible oxidase synthetize (iNOS), nitric oxide (NO), interleukin-12 (p70) (IL-12), tumor necrosis factor alpha (TNF-α), and MCP-1. Cell signaling pathways were also modified through the action of theasinensin A – downregulation of MAPK/ERK kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling through a direct binding to MEK-ERK was proofed (Weerawatanakorn et al. 2015b). These findings were confirmed in the mouse paw edema model – theasinensin A inhibited the production of IL-12 (p70), TNF-α, and MCP-1 and attenuated the inflammation in mouse paw edema caused by lipopolysaccharide LPS (Hisanaga et al. 2014).

19.4.2.3 Anti-obesity Activity

Oolong tea was proofed to possess significant activity toward the reduction of weight. Although such experiments were not conducted for particular theasinensins, a study performed on high-fat diet-induced obese mice revealed reduction of fat production through the inhibition of pancreatic amylase. This caused significant decrease in lipid absorption and increased lipid excretion into the feces. Another study demonstrated that the supplementation with oolong tea reduced the concentration of LDL-cholesterol, total cholesterol, and triacylglycerols in serum plasma in rats and decreased their weight. Oolong tea was also proofed to strongly decrease the levels of triacylglycerols in comparison to green tea. Oolong tea was also proofed to increase plasma levels of SOD and weight ratios of the liver to epididymal adipose tissue in rats. The fact that oolong tea reduces the levels of MCP-1, a protein which concentration is positively associated with higher incidence of obesity, indicates that active constituents of oolong tea may play a beneficial role in the prevention of this disease (Weerawatanakorn et al. 2015b).

19.4.2.4 Anticancer Activity

Several studies reported that oolong tea may be characterized by strong cancer-chemopreventive properties, similar to green or black teas. Some authors suggested that catechins play a dominant role and were mostly responsible for this activity.

However there are not much research performed to investigate anticancer activity of theasinensins. In theory since initiation and/or prolongation of different types of cancers is connected with inflammation and generation of free radicals, significant anti-inflammatory and antioxidant properties of theasinensins may play a crucial role in cancer prevention caused by oolong tea and its active constituents. In vitro studies revealed molecular basis of potential anticancer activity of TSs. Using two cell lines – human histolytic lymphoma (U937) and acute T-cell leukemia (Jurkat) – it was shown that theasinensin A, extracted from oolong tea, induced apoptosis through the release of cytochrome c into the cytosol and activation of caspase-9 and caspase-3, loss of mitochondrial transmembrane potential, and elevation of free radicals production (Pan et al. 2000). Maeda-Yamamomota and co-workers investigated potential role of tea polyphenols in the inhibition of metastasis using human fibrosarcoma HT1080 cells. The study revealed weak antimetastatic activity of theasinensin D in comparison to theaflavin and ECG, which was considered as the most active (Maeda-Yamamoto et al. 1999).

There are limited data on anticancer activity of particular theasinensins in the in vivo model. Study performed by Zhang and co-workers revealed that oolong tea, similar to black and green teas, induced apoptosis and arrested cell cycle in male Donryu rats (Zhang et al. 2000). However no particular theasinensins were used in this experiment, so it is not obvious if this activity was caused by catechins, TSs, or other active compounds present in oolong tea extract. Oral administration of theasinensin A in mice with carbon tetrachloride (CCl4)-induced fibrosis relieved liver injury and ameliorated liver functions. Immunohistological staining has shown that supplementation with theasinensin A reduced collagen deposition and inhibited inhibition of transforming growth factor β (TGF-β). It was concluded that theasinensin A may be considered as a potential bioactive compound from oolong tea, which prevents liver fibrosis (Hung et al. 2017). Based on previous studies, it could be concluded that chemopreventive effects of oolong tea extracts are due to its anti-inflammatory, antimutagenic, and anti-genotoxic activity; however there are still not many experiments performed on pure theasinensins in the in vivo model (Weerawatanakorn et al. 2015b).

19.4.2.5 Hypoglycemic Effect

Aqueous extract from oolong tea, similar to green and black teas, was proofed to increase insulin activity by 15-fold in an epididymal fat cell assay (Anderson and Polansky 2002). No impact of oolong tea on Na^+-dependent glucose cotransporter (SGLT1) was shown in frogs (Xenopus oocytes) compared to tea catechins (Hossain et al. 2002). A study performed on 20 people (women and men, average age of 61.2 years) suffered from diabetes since almost 5 years, which have obtained 1500 mL of oolong tea per day for 30 days, revealed significant decrease of plasma glucose from 229 to 162.2 mg/dL and fructosamine from 409.9 to 323.3 μmol/L. Water was used as control. It was also proofed that the combination of oolong tea and hypoglycemic drugs can cause stronger effects toward reducing

blood glucose than taking the drugs alone (Hosoda et al. 2003). It was indicated that crucial compounds of oolong tea responsible for this activity are theasinensins. Studies performed in mice and rat models revealed that theasinensin A reduces serum glucose levels by over 30%, inhibits glucose production and absorption in the intestine through suppression of α-glucosidase activity, and induces antihyperglycemic responses by suppressing fat absorption. Other studies performed using cell lines have shown that theasinensins A and B can promote glucose cell uptake and improve insulin resistance by regulation of GLUT 4, which is insulin-regulated glucose transporter present predominantly in adipocyte and striated muscle tissue. It was also revealed that this mechanism differs from EGCG, which can also regulate the activity of GLUT 4 (Weerawatanakorn et al. 2015b).

19.4.2.6 Prevention of Cardiovascular Diseases

There are epidemiological studies which have proofed beneficial effects of oolong tea consumption toward the cardiovascular system. An epidemiological study was performed on 1507 subjects from Taiwan. The study revealed that 600 were habitual tea drinkers (green or oolong tea) who consumed at least 120 mL of tea infusion per day during the period of 1 year or more. The study revealed that in the group of habitual tea drinkers, the risk of developing hypertension was reduced by 46% for those who drank 120–599 mL of tea per day and was further reduced by 65% for those who drank 600 mL or more. Unfortunately the study did not separate people who drink green from oolong tea (Yang et al. 2004). Since high-fat and cholesterol diet was proved to increase the risk of atherosclerosis and coronary heart disease, activities leading to decrease lipid intake and absorption and causing higher energy expenditure (EE) are considered crucial in decreasing the risk of cardiac diseases. Hsu and co-workers have shown that the consumption of polyphenol-enriched oolong tea increases fecal lipid excretion in patients taking high-fat diet (Hsu et al. 2006). Moreover, a study performed on 11 healthy Japanese women proved beneficial effects of oolong tea drinking for the increase of energy expenditure. This experiment showed that oolong tea increased EE by 10% and green tea by 4%. Since green tea contains on average two times more caffeine and EGCG in comparison to green tea, the authors suggested that polymerized polyphenols present in oolong tea, of which theasinensins are the major fraction, are responsible for this activity (Komatsu et al. 2003). Study performed by Rumpler and co-workers revealed similar results, oolong tea consumption increased EE and fat oxidation. Its consumption can help to maintain low body weight and reduce the risk of atherosclerosis and hypertension (Rumpler et al. 2001). Oolong tea was proved to reduce the atherogenic index and increased the HDL-total cholesterol ratio in rats fed with high-fat diet (Yang and Koo 1997). Above findings confirmed beneficial effects of oolong tea consumption on the prevention of cardiovascular diseases; however there is still lack of data on the bioactivity of theasinensins toward heart diseases which could be found in the literature.

19.4.2.7 Antimicrobial Activity

Several studies have reported the activity of oolong tea extracts or particular theasinensins toward the inhibition of pathogen growth. Among them the following strains may be mentioned: *Streptococcus sobrinus* 6715, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella* sp., *S. mutans MT8148R*, and *Staphylococcus aureus* (Weerawatanakorn et al. 2015b). All of these studies were performed in vitro, and no in vivo studies for antimicrobial activity of theasinensins can be found in the scientific literature at the moment.

19.4.3 Conclusions

Theasinensins are still poorly understood. There is a need to continuing studies on the content of particular TSs in black and oolong teas. As for now there are some reports which showed that the major theasinensin is theasinensin A, which concentration was higher compared to theasinensins B and D. It was also proved that the concentration TSs in oolong tea is 0.65% and was much higher compared to green tea (0.05%). More studies regarding the activity of particular theasinensins in the in vivo model, as well as scientific data regarding their absorption, metabolism, and microbial degradation, are needed to extend the knowledge on these polymerized polyphenols.

19.5 Patents

The screening for patents regarding polymeric polyphenols from tea gives over 7,000 of results. The biggest part regards theaflavins (almost 5,000 results), which are the best-studied from all condensed tea polyphenols. The majority of these inventions disclose the production process, biological activity, and usage as food components or dietary supplements. The table below shows selected patents regarding theaflavins, thearubigins, and theasinensins (Table 4).

19.6 Summary

Tea contains a large number of active substances. Its positive influence on the human organism is known since centuries. During production process of fermented and semi-fermented teas (mainly black and oolong), simple catechins are oxidized to form polymeric polyphenols. These substances have not been fully understood yet, and studies on understanding their structure, synthesis pathways, and biological properties are still carried out. Currently, it is known that they have many beneficial

Table 4 Selected patents regarding polymeric polyphenols from tea

Patent number	Country	Title	Brief description	Year
Theaflavins				
CN101096693A	China	*Method for preparing theaflavin and thearubigin from fresh green tea*	The invention discloses a making method of theaflavin and Congo red element from fresh green tea, without using toxic and harmful organic solvents	2007
US7157493B2	USA	*Methods of making and using theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin 3,3′-digallate and mixtures thereof*	The invention discloses methods of making a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin 3,3′-digallate, usage in pharmaceutical industry and production of dietary supplements	2003
CN1729807A	China	*Process for preparing keemun black tea with highly concentrated theaflavin and thearubigins*	The invention discloses a process for preparing keemun black tea rich in theaflavins and thearubigins	2004
US5532012A	USA	*Process for preparation of purified tea components using preconcentration by cream separation and solubilization followed by medium pressure chromatography and/or preparative HPLC*	The invention describes a method of obtaining selected tea polyphenols, including theaflavins, using liquid/liquid extraction and chromatographic techniques	1995
CN101808529A	China	*Process for extracting theaflavins from tea*	The invention describes process of extraction of theaflavins from tea leaves using water and ethyl acetate simultaneously or sequentially. As the result ethyl acetate extract rich in theaflavins is obtained	2007
US20090098224A1	USA	*Metabolic-enhancing properties of theaflavins and thearubigins*	The invention discovered that theaflavins and thearubigins reduce appetite and adipose tissue, increase metabolism and energy expenditure, and enhance lean body mass	2007

(continued)

Table 4 (continued)

Patent number	Country	Title	Brief description	Year
CN102524536A	China	*Application of theaflavin as feed additive and corresponding feed*	Application of theaflavin as the food additive in the concentration of 0.015–0.045% in different foods, e.g., fish meal, bean pulp, or peanut meal	2011
JP2009268420A	Japan	*Functional food composition*	The purpose of the invention is to produce a functional food with an inhibitory potential toward α-glucosidase and lipase containing different theaflavins in the concentration of 2–10%	2008
Thearubigins				
CN101473880B	China	*Method for extracting and separating thearubigins from black tea*	The invention describes method for separation of thearubigins from black tea using simple extraction equipment. The biggest advantages of the proposed method are low costs, high efficiency, and low impact on the environment	2009
CN101518597A	China	*Application of thearubigins to pharmacy*	The invention describes a new purpose of thearubigins in the field of pharmacy as natural products which can be used for treating or preventing type II diabetes, lipogenous diabetes, and adiposis. Moreover the invention proves that thearubigins have no toxic side effects and are safe and reliable to take	
CN108522674A	China	*Processing method of high-quality black tea*	The invention describes the production process of high-quality black tea. One of the advantages is the promotion of theaflavin and thearubigin	2018

(continued)

Table 4 (continued)

Patent number	Country	Title	Brief description	Year
			formation through the increased oxidation of simple catechins by polyphenol oxidase and beta-D-glucosidase	
CN106509478A	China	*Feed additive for improving immunity and antioxidation ability of river crabs and preparation method thereof*	Composition of food additive, containing among others 20–40 parts of thearubigin, for improving the immunity and antioxidation ability of river crabs	2016
CN107156310A	China	*Instant health-care antiaging soybean milk powder*	The invention relates to the technical field of production of instant health-care antiaging soybean milk powder containing among others thearubigin	2017
CN105285189A	China	*Making method of black tea cakes*	The invention describes the production of black tea cakes rich in thearubigins with the reduced amounts of theaflavins	2015
CA2762665A1	Canada	*A prebiotic composition comprising thearubigin*	The invention proposes the usage of black tea polyphenols (containing 82% of thearubigins) as a prebiotic and/or for the treatment or prevention of conditions associated with poor gut health or low immunity	2010
CN106578219A	China	*Method for highly efficiently purifying thearubigins prepared through chemical oxidation of tea polyphenol*	The invention describes purification methods of thearubigins obtained through chemical oxidation of tea polyphenols. The method removes sodium chloride with high efficiency and allows to obtain high-purity thearubigins	2016
Theasinensins				
KR101296418B1	South Korea		The invention provides food and beverage	2005

(continued)

Table 4 (continued)

Patent number	Country	Title	Brief description	Year
		Lipase inhibitor containing theasinensins	containing lipase inhibitor based on a mixture of theasinensins (A, B, and D)	
US20110064851A1	USA	*Method of producing fermented tea drink rich in theaflavins*	The invention describes the method for preparing a fermented tea drink rich in theaflavins and theasinensins A and B, characterized by little bitterness and astringency, characterized by excellent taste and sweetness	2009
JP2010138103A	Japan	*Method for producing theasinensin*	The invention presents method for producing theasinensin by oxidizing EGC and/or EGCG in the presence of a nonhomogeneous catalyst and then reducing the obtained product. The method provides higher efficiency compared to the conventional methods	2008
CN104621291A	China	*Method for extracting and preparing theasinensin from tea leaves*	The invention discloses solid-liquid extraction of theasinensins from tea leaves using acetone solution	2015
JP2010189321A	Japan	*Cholesterol-lowering agent*	The invention describes cholesterol-lowering agent based on natural products, which contains theasinensin	2009

effects on the body and in the future they can be used in the pharmaceutical and food industry on a much larger scale than at present.

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Stilbenoids in Grapes and Wine

20

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Abstract

Stilbenoids are naturally occurring polyphenols, found in several edible plants, such as berries, peanuts, and grapes, which have been attracting increasing interest owing to their potential benefits for human health, namely, to prevent and treat chronic diseases related with aging. They can be constitutively expressed or biosynthesized as phytoalexins. Structurally characterized by a C6–C2–C6 scaffold, they can be found as monomers, dimers, trimers, or very complex oligomers. In this chapter, the focus will be on dietary stilbenoids found in the *Vitis vinifera*, namely, in grapes and wine, which are the principal dietary source of stilbenes. Among them, *trans*-resveratrol is the most extensively investigated stilbene. The main bioactive constituents, bioavailability and metabolism, the *in vivo* biological properties of the major constituents, and their role in human health, application in food, as well as issues related with safety and marketed and patented products will be also discussed.

Keywords

Stilbenes · *Vitis vinifera* · Wine · Polyphenols · Oligostilbenes · *Trans*-resveratrol · *In vivo* studies · Bioavailability · Metabolism · Clinical trials

20.1 Introduction

Stilbenes or stilbenoids are plant-derived polyphenols, occurring in several plant families being particularly abundant in Gnetaceae, Pinaceae, Cyperaceae, Fabaceae, Dipterocarpaceae, and Vitaceae (Rivière et al. 2012). They have been attracting increasing interest due to their diverse biological activities and potential benefits for human health, namely, cardioprotection, neuroprotection, antidiabetic properties, anti-inflammation, and cancer prevention and treatment, making them important lead compounds in drug discovery and development. An important example of this is *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene, **1**), commonly known as resveratrol, which has shown several biological activities, including strong antioxidant effects, thus providing cardiovascular protection by reducing oxidative stress. The cardioprotective effects of resveratrol have been connected to the "French Paradox." The term is related to the paradoxical epidemiological observation that French population has a low incidence of coronary heart disease, despite the high intake of saturated fats. It was hypothesized that French paradox might be owing to the consumption of red wine, which may contradict the negative effects of a diet rich in saturated fat. This conclusion fostered the interest in the overall phenolic constituents of red wine and particularly in resveratrol that has been extensively investigated (Akinwumi et al. 2018).

In plants, stilbenoids can be constitutively expressed or can be synthetized as phytoalexins in response to fungal and bacterial pathogens and/or to other environmental stress factors (Xiao et al. 2008). This class of secondary metabolites derives from phenylpropanoid and acetate-malonate pathway with a chemical structure

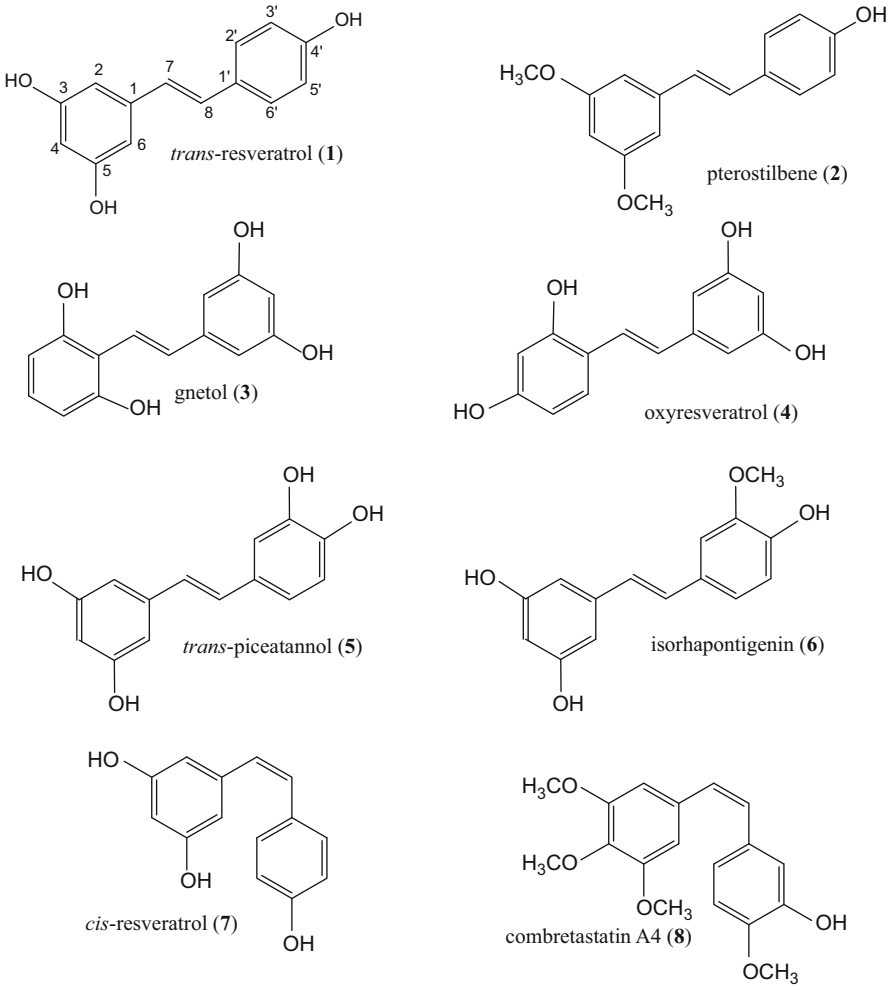

Fig. 1 Examples of monomeric *trans*-stilbenes (**1**–**6**) and *cis*-stilbenes (**7** and **8**) with hydroxyl and methoxyl substituents

based on a C6–C2–C6 backbone, where two phenyl rings are joined by an ethylene bridge. They can assume both a *trans* (*E* isomers, **1**–**6**, Fig. 1) and a *cis* configuration (*Z* isomers, **7** and **8**, Fig. 1), which elicits different pharmacological activities, being the *trans* isomer the most common and stable configuration in naturally occurring stilbenes. Structural variations in stilbenoids include different substituent patterns in the aryl groups, which may bear different hydroxyl, methoxyl, prenyl, or geranyl moieties and may also appear as glycosides (Figs. 1 and 2). They can be found as monomers and as very complex oligomers, which may result from oxidative coupling of monomeric stilbenes through C–C or C–O–C bonds of resveratrol (**1**), oxyresveratrol (*trans*-2,3′,4,5′-tetrahydroxy-stilbene) (**4**), piceatannol *(trans*-

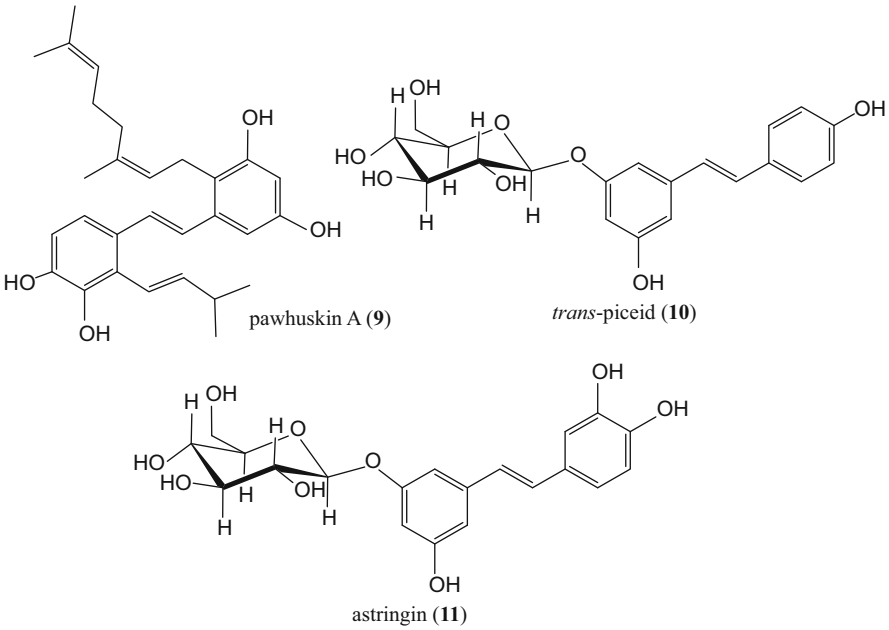

Fig. 2 Examples of monomeric stilbenes with prenyl, geranyl, and glycosyl substituents

3,3′,4,5′-tetrahydroxy-stilbene) (**5**) or isorhapontigenin (*trans*-3,4′,5-trihydroxy-3′-methoxystilbene) (**6**) (Pawlus et al. 2012; Rivière et al. 2012; Shen et al. 2009).

Vitis vinifera L. (Fig. 3) belongs to the Vitaceae family and is the main dietary source of stilbenes, which are consumed in the form of grapes, grape juice, and wine. The therapeutic and health-promoting properties due to the consumption of grapes and related products have been attributed to the presence of a complex combination of polyphenolic compounds, which includes not only monomeric stilbenes such as resveratrol (**1**) but also simple phenolics and flavonoids among others, being the latter the largest group (Georgiev et al. 2014).

In this review, the focus will be on the beneficial health effects of the dietary stilbenoids found in the *V. vinifera*. The activities of the main stilbenoids, found in grapes and wine, on relevant animal assays and clinical trials will be discussed in terms of bioavailability and metabolism, principal biological activities, and their role in human health. Application in food, as well as issues related with safety and marketed and patented products, will be also discussed.

20.2 Stilbenes in *Vitis vinifera*

Structurally characterized by a C6–C2–C6 scaffold, stilbenes are secondary metabolites derived from a mixed biosynthetic pathway (Fig. 4). In this way, one *p*-coumaroyl-CoA starter molecule, derived from the shikimate pathway, is

Fig. 3 *Vitis vinifera* (Pixabay 2019; Wikimedia commons 2019)

condensed to three malonyl-CoA molecules derived from the acetate pathway, giving rise to a polyketide chain (Dewick 2009). Stilbene synthase enzyme catalyzes the folding and subsequent cyclization through an aldol condensation reaction that generates the basic skeleton of stilbenes, represented by resveratrol (**1**). Further methylation, glucosylation, and prenylation reactions give rise to a myriad of stilbenoid derivatives.

Stilbenoids, found in several edible plants, such as berries, peanuts, and grapes, are considered promising compounds to prevent and treat chronic diseases related with aging (Khawand et al. 2018). More than 1000 stilbene derivatives have been identified (Pawlus et al. 2012), ranging from monomers to octamers and different substituent patterns. In Vitaceae family, they are found in several species, including *V. vinifera*, the most important species for wine production. In fact, red wine and grapes are the main dietary source of the most known and studied stilbene resveratrol (**1**). Resveratrol (**1**) and its derivatives, with several patterns of oligomerization and glycosylation, are also found in the whole plant, such as leaves, canes, wood, and roots of the grapevine and in larger amount than in grape berries (Gabaston et al. 2017).

Oligomeric stilbenes were previously classified into two main groups, according to the presence or not of at least one five-membered oxygen-containing heterocycle (Sotheeswaran and Pasupathy 1993). Owing to its limitation, there have been several attempts to improve this classification, by dividing first oligomeric stilbenes according to their monomeric moieties and heterogeneous coupling and then further classifying these based on the presence (group A; e.g., ε-viniferin, **12)** or absence (group B; e.g., pallidol, **13**) of oxygen-containing heterocycles (Fig. 5) (Shen et al. 2009).

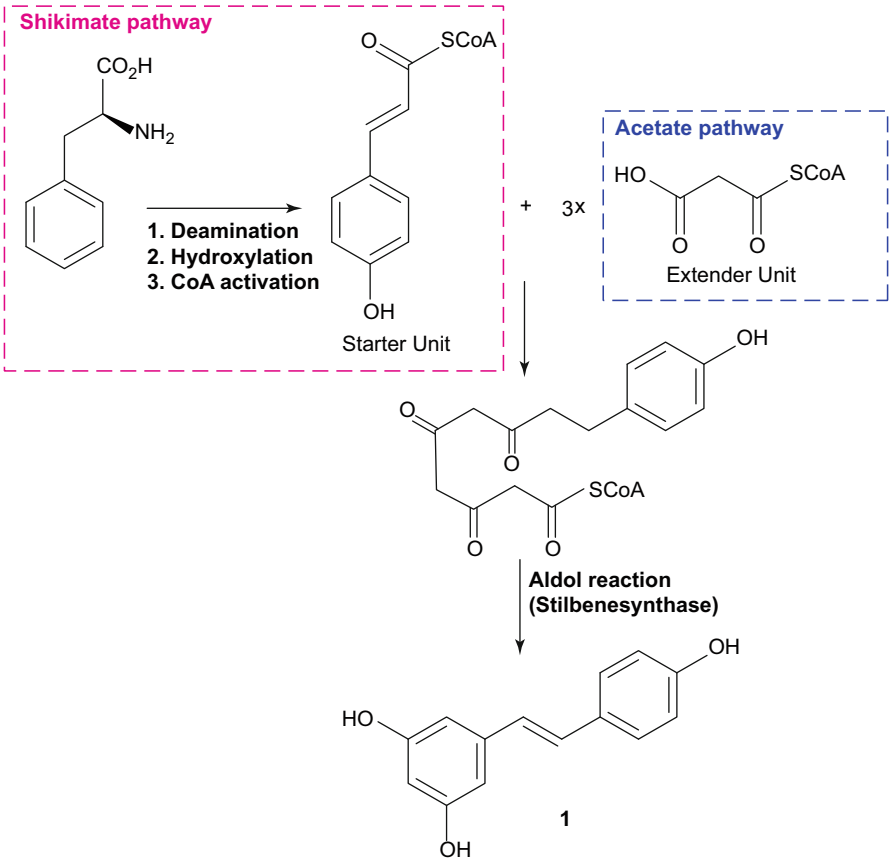

Fig. 4 Biosynthesis of stilbenes

Most of the stilbenes found in *Vitis* species as monomers are methoxylated and predominantly glycosylated derivatives of resveratrol (**1**) and piceatannol (**5**). The monomers can be found as *trans* and *cis* isomers such *trans*- and *cis*-piceid [*trans*-(**10**) and *cis*-resveratrol-3-*O*-β-D-glucopyranoside], pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxy-stilbene) (**2**), and astringin (**11**), the 3-β-D-glucoside of piceatannol (Figs. 1 and 2). In *V. vinifera*, the number of these stilbenes is lower in wine than in grapes (ISVV database 2017).

Concerning the dimeric forms of stilbenes found in wine, most of them bear resveratrol (**1**) as monomer. They are essentially derivatives of viniferin and pallidol (**13**), being the most frequent ε-viniferin (**12**) (Fig. 5), the major oligomer found in *Vitis* species. While several trimers and tetramers (**13**) have been also found in *Vitis* species, including in different plant parts of *V. vinifera*, only one of these oligostilbenes, namely, the tetramer hopeaphenol (**13**, Fig. 6), has also been reported in wine. Two pentamers were isolated from *V. amurensis* roots, but there is no report for the presence of this type of oligomers in *V. vinifera*. Conversely, more recently,

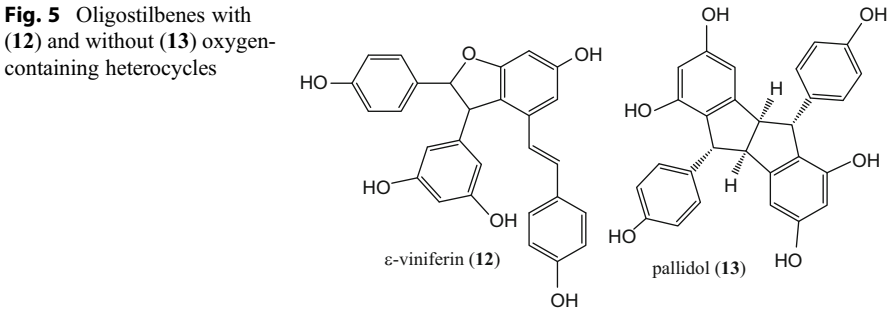

Fig. 5 Oligostilbenes with (**12**) and without (**13**) oxygen-containing heterocycles

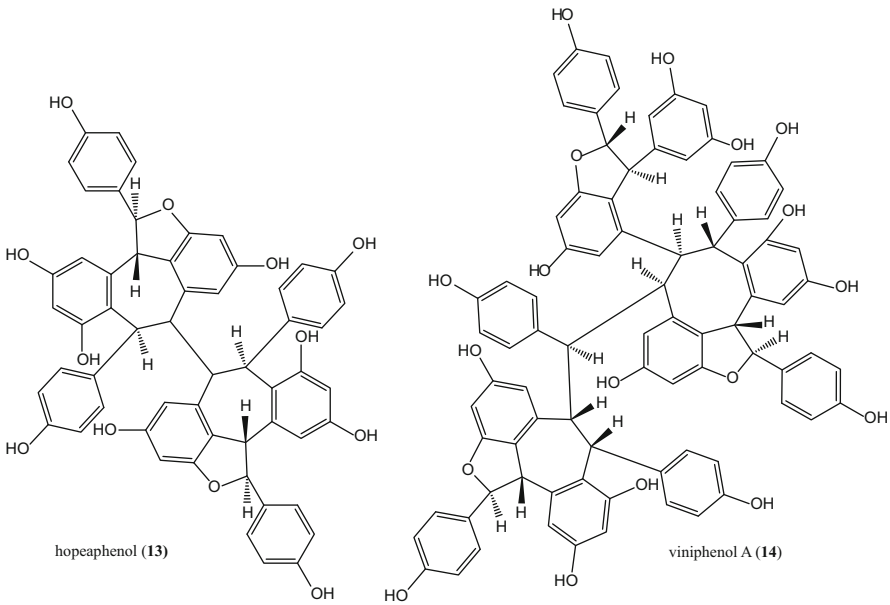

Fig. 6 Examples of oligostilbenes found in *V. vinifera*

the resveratrol hexamer viniphenol A (**14**) (Fig. 6) was isolated from the vine stalks of *V. vinifera* (Pawlus et al. 2012; Shen et al. 2017).

20.3 Bioavailability and Metabolism

In the last two decades, extensive studies have been conducted in order to find out the biological effects of stilbenes. Concomitantly, bioavailability and other pharmacokinetic preclinical studies have been performed in animal models and humans. In this regard, the most studied stilbene is resveratrol (**1**), although in recent years, pterostilbene (**2**) and piceatannol (**5**) have also begun to be explored. Unfortunately,

and notwithstanding all the promising biological effects reported for several disease models, the pharmacokinetic properties and bioavailability of these stilbenes are not so satisfactory, a fact that may limit their application in clinical use.

Despite its low water solubility (< 0.05 mg/mL), resveratrol (**1**) is rapidly absorbed at the small intestine by passive diffusion and undergoes a promptly and extensive phase II metabolic reactions catalyzed by uridine 5′-diphosphoglucoronyltransferases (UGTs) and sulfotransferases (SULTs) in the intestinal epithelial cells and liver. It is reported that after an oral administration of resveratrol, its plasma half-life is about 8–14 min and consequently only about 1–2% of its free form may be found in plasma (Dvorakova and Landa 2017). Resveratrol-3-*O*-glucoronide (**15**), resveratrol-4′-*O*-glucoronide (**16**), their sulfate analogues (**17**, **18**), and resveratrol-3,4′-*O*-disulfate (**19**) have been reported as the major phase II metabolites (Fig. 7). The conjugation to sulfates and glucuronic acid increases water solubility and allows the excretion by the kidneys, being these metabolites mainly eliminated in urine (Singh et al. 2015).

On the other hand, *in vitro* studies using human liver microsomes demonstrated the formation of two tetrahydroxylated resveratrol metabolites, piceatannol (**5**) and another one that was putatively identified as 3,4,5,4′-tetrahydroxystilbene (**20**), which resulted from phase 1 metabolic reactions. This study suggested the major role of cytochrome P450 (CYP), particularly the isoform CYP1A2 in this metabolic

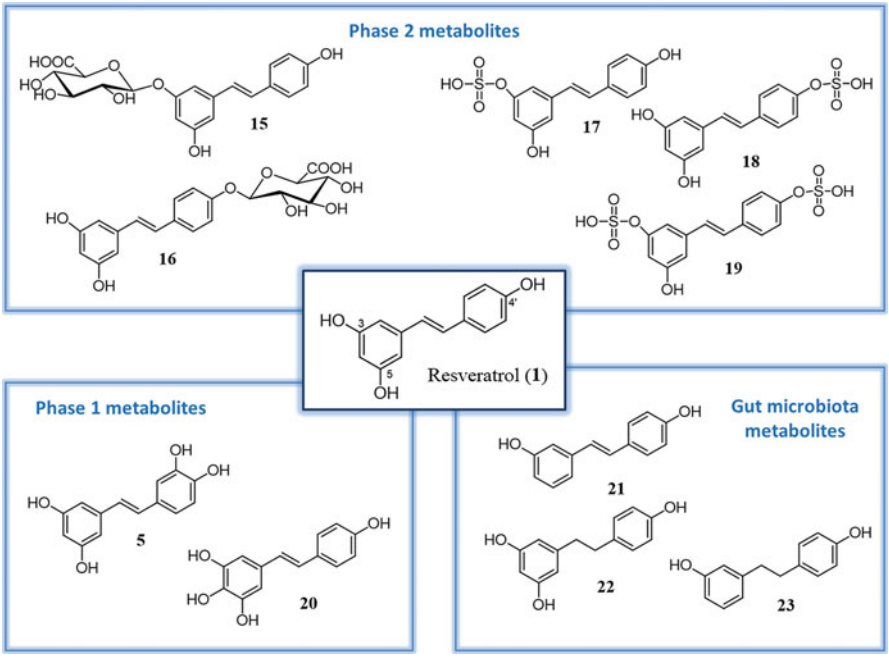

Fig. 7 Resveratrol metabolites resulting from phase 1 and 2 metabolic reactions and microbial biotransformation in gut

process (Piver et al. 2004). Other metabolites have been identified, in fecal samples, such as 3,4′-dihydroxy-*trans*-stilbene (**21**), dihydroresveratrol (**22**), and 3,4′-dihydroxybibenzyl (lunularin, **23**), highlighting the important function of gut microbiota in resveratrol metabolism (Fig. 6) (Wang and Sang 2018). The extensive metabolism of resveratrol decreases circulating levels of the free molecule; thus, it is suggested that biological effects could be also attributed to resveratrol metabolites or probably to the enterohepatic recycling involving biliary secretion, which may result in some deconjugation by gut microflora in the small intestine and reabsorption of the original molecule (Akinwumi et al. 2018).

However, the absorption and metabolic processes appear to be dependent on different factors, such as specific inter-individual variability, administration routes and doses, species, gender, disease status, and mode of consumption (fasting or with food or wine). Several studies carried out both on animals and humans compared the pharmacokinetics of resveratrol regarding different doses and treatment regimens and administrations routes (oral and intravenous). To study the influence of the administration routes on resveratrol pharmacokinetics in humans, ^{14}C-labeled resveratrol was administered orally or intravenously with doses of 25 and 0.2 mg, respectively (Walle et al. 2004). A high absorption rate was observed followed by an extensive biotransformation in both types of administration, resulting in only trace amounts of free resveratrol in the systemic circulation. After an oral dose of 25 mg, a total radioactivity of 491 ± 90 ng/mL (resveratrol and metabolites) was reached at about 1 h after the dose. Curiously, at around 6 h after consumption, a second peak plasma total radioactivity was observed, which was suggested to be a result of the enterohepatic recirculation of conjugated metabolites. The urinary excretion was assessed and indicated at least 70% of absorption and a recovery of total radioactivity ranging from 53.4% to 84.9% in urine after oral consumption. Similar results were obtained after the i.v. dose of 0.2 mg of ^{14}C-labeled resveratrol. One hour after the bolus injection, a rapid decrease of total radioactivity was observed on plasma, but the second plasma peak was not detected (Walle et al. 2004).

In a study performed in healthy volunteers that took orally low doses of resveratrol (5 or 50 mg), the main metabolites found in plasma were glucuronides. On the other hand, when high doses were administered (> 250 mg), resveratrol-3-*O*-sulfate (**17**) was the main identified metabolite. Interestingly, in plasma samples collected 11–21 h after intake, the main identified metabolite was the derivative conjugated with both glucuronide and sulfate moieties (Wang and Sang 2018).

In order to study the influence of different doses of resveratrol, a pharmacokinetic study was conducted by orally administering single doses of 0.5, 1, 2.5, or 5 g, in 10 healthy volunteers per dose level (Boocock et al. 2007). The rapid absorption of resveratrol was corroborated in this study, achieving a peak plasma concentration in the 0.83 h–1.5 h post-dose. Peak plasma levels of resveratrol at the highest dose were 0.54 ± 0.38 μg/mL achieved in the 1.5 h post-dose. Two monoglucuronides and resveratrol-3-*O*-sulfate (**17**) were the main identified metabolites, and their concentration exceeds those of resveratrol by up to 20-fold. The study also concluded that excretion rates were highest in the 4 h post-dose, and when the lowest dose was consumed, 77% of all urinary species were excreted within this period. In the 24 h

post-administration of the 0.5 g dose level, the amount of resveratrol excreted in the urine was below 0.04% of the dose, whereas urinary excretion of the three resveratrol conjugates ranged from 0.51% (one of the glucuronides) to 11.4% of the dose (resveratrol-3-*O*-sulfate, **17**). This study proved that only traces of resveratrol could be found in plasma even after high-dose consumption, suggesting that ingestion of resveratrol equivalent to the amount contained in several hundred bottles of red wine is not enough to elicit the biological effects associated with this compound (Boocock et al. 2007). The concentrations used in *in vitro* or *in vivo* studies with resveratrol supplements are too high to be reached in the organism after wine consumption. A very interesting research was conducted aiming at study resveratrol bioavailability after a moderate consumption of red wine (300–600 mL) associated with fasting and different meals: a standard meal and meals with high and low amount of lipids (Vitaglione et al. 2005). It appears that resveratrol bioavailability is not influenced by fasting, the type of meal, or the amount of lipid content. Moreover, it was demonstrated that resveratrol absorption and metabolism after wine consumption are highly variable, being found in the serum of roughly half of the subjects in free or in glucuronidated form and in very low concentrations. Other studies were carried out to study the matrix effects on the absorption and bioavailability. Theoretically, the absorption rate of resveratrol may increase when administered with a matrix that promotes its solubility (Peng et al. 2018). These researches comprised the moderate consumption of red wine, grape juice, resveratrol tablets, and different doses of resveratrol dissolved in different matrices, including white wine, grape juice, vegetable shakes, and diluted ethanol or whisky. Controversial results were obtained since some studies concluded that there were no significant differences wherever the type of matrix, whereas other studies claimed that absorption and bioavailability of resveratrol were higher when pure compound was administered (Peng et al. 2018).

It was also found that there is no significant difference between a single high-dose treatment and repeated administrations of resveratrol. In a double-blind, randomized, placebo-controlled study to investigate the multiple-dose pharmacokinetics, four groups of 10 healthy volunteers received 25, 50, 100, or 150 mg of resveratrol, six times/day (every 4 h), for 2 days (Akinwumi et al. 2018; Wang and Sang 2018). Similarly to other studies, peak plasma concentrations were reached at 0.8–1.5 h post-dose. Despite the multiple administrations, the degree of resveratrol accumulation in plasma was not significant even for the highest dose. However, high inter-individual variability and circadian variations were observed, being the residual plasma concentration highest after morning administration and lowest during the night (Akinwumi et al. 2018).

Although much less studied than resveratrol, the bioavailabilities of pterostilbene (**2**) and piceatannol (**5**) are nowadays beginning to be unraveled. In comparison with resveratrol, pterostilbene (**2**) has two methoxyl groups located at C-3 and C-5, which confer higher lipophilicity improving the cell permeability and increasing oral absorption. Moreover, pterostilbene (**2**) is considered to be more metabolically stable since it has only one free hydroxyl group available for phase 2 metabolic reactions; therefore, pterostilbene-4′-*O*-glucuronide (**24**) and pterostilbene-4′-*O*-sulfate (**25**) were characterized as its main metabolites (Fig. 8). Consequently, it seems that

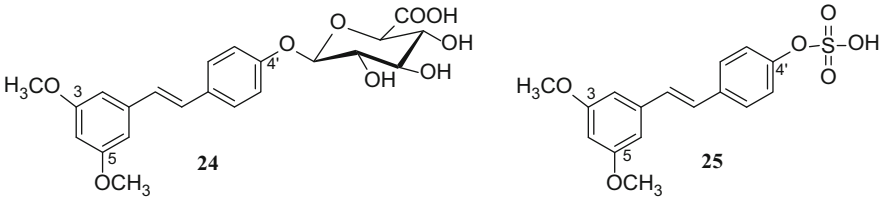

Fig. 8 Main metabolites of pterostilbene (**2**)

pterostilbene is tenfold more bioavailable than resveratrol (Akinwumi et al. 2018; Peng et al. 2018; Wang and Sang 2018).

Regarding piceatannol (**5**), the vast majority of studies is yet rather limited to cells and animal models. *In vitro*, piceatannol is considered to be a product of CYP450 metabolism of resveratrol. This was corroborated by *in vivo* studies, where piceatannol was also detected in plasma and liver of athymic mice, 5 min after oral administration of resveratrol (Kershaw and Kim 2017). Concerning piceatannol metabolism, *in vitro* studies using human liver cytosol and microsomes described the existence of sulfation and glucuronidation metabolic reactions and identified piceatannol disulfate, two monosulfated, and three monoglucuronide metabolites, although the exact location of the conjugated groups was not possible to be determined (Fig. 9) (Kershaw and Kim 2017).

After i.v. administration in rats, glucuronides seem to be the main metabolites. After intragastric administration, a piceatannol-monoglucuronide was found to be the most abundant metabolite, but other ones were also observed, such as sulfated and methylated metabolites, in particular isorhapontigenin (**6**) and rhapontigenin (**26**) (Fig. 9). Rhapontigenin (**26**) was also found in urine, as well as piceatannol mono- and diglucuronides, *O*-methylpiceatannol-monoglucuronide, and *O*-methylpicetannol-monosulfate. In contrast to piceatannol (**5**), methylated compounds were not found as metabolites of resveratrol (Kershaw and Kim 2017).

As mentioned above, oral bioavailability of resveratrol is highly compromised by its extensive metabolism and elimination rate (Peng et al. 2018). Currently, these factors are trying to be overcome by using drug delivery systems employing nanotechnology for resveratrol encapsulation, which comprise liposomes, polymeric nanoparticles, solid lipid nanoparticles, lipospheres, and cyclodextrins (Summerlin et al. 2015). Studies performed in animal models showed that these novel nanocarriers possess several advantages, including improved solubility, physicochemical stability, and protection from metabolic reactions thus overcoming first-pass effects. Moreover, such nanoformulations could enhance transport across the membranes, increasing concentration of resveratrol in tissues such as the brain, liver, and kidney, enabling targeted and controlled drug release (Siddiqui et al. 2015).

Recently, a clinical trial was designed by Calvo-Castro et al. (2018) in order to assess the improvement of the oral bioavailability of resveratrol from vineatrol by micellar solubilization (registration number: NCT02944097). Vineatrol is a standardized ethanolic extract of grapevine shoots that contains 33.3% resveratrol

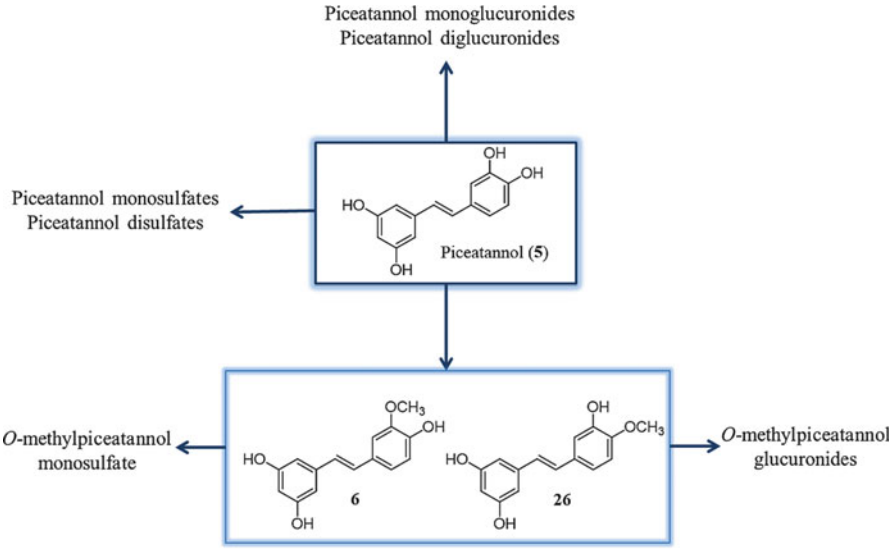

Fig. 9 Piceatannol metabolites

monomers and oligomers, including 5.8% resveratrol (**1**), 14.5% *trans*-ε-viniferin (**12**), and minor amounts of ampelopsin (2.4%), hopeaphenol (**13**) (2.2%), piceatannol (**5**) (0.5%), R2-viniferin (1.2%), R-viniferin (1.8%), miyabenol C (2.3%), and E-omega-viniferin (2.6%). Thus, twelve healthy volunteers (six women, six men) randomly ingested a single dose of 500 mg vineatrol (30 mg resveratrol, 75 mg ε-viniferin) as native powder or liquid micelles (placebo uncontrolled). Oral ingestion of the soluble vineatrol micelles improved the relative systemic bioavailability with of total resveratrol when compared to the native powder, without any indication of adverse effects. The mean maximum total plasma resveratrol concentrations after the intake were 300 and 28 nmol/L, respectively. The authors conclude that a micellar microemulsion provides a safe delivery of resveratrol in order to reach relevant bioactive concentrations to systemic circulation (Calvo-Castro et al. 2018).

20.4 Bioactivities of Stilbenoids: *In Vivo* Studies

Considering the bioactivities of stilbenes, using *in vivo* studies, there are several works describing the large benefit effects on different human health areas, such as hypolipidemic action, obesity, type 2 diabetes, and dementia prevention and anti-inflammatory, antioxidant, antiaging, anticancer, depigmentation, and cardiovascular effects. Several works, focusing on wine and grapes, have been reviewed regarding stilbenoids *in vivo* studies, emphasizing not only the well-studied resveratrol (**1**) but also pterostilbene (**2**), oxyresveratrol (**4**), piceatannol (**5**), piceid (**10**), (Figs. 1 and 2), and oligostilbenes (Table 1).

Table 1 Bioactivities of stilbenes from wine and grapes and main conclusions of recent works regarding animal studies

Stilbenes	Animal study	Main conclusions	References
Resveratrol (**1**), pterostilbene (**2**), oxyresveratrol (**4**) piceatannol (**5**), and *cis*-piceid (**27**)	Bioactivities review which includes animal studies in mice	Stilbenoids have different biological properties such as cardioprotection, neuroprotection, antidiabetic properties, depigmentation, anti-inflammation, cancer prevention and treatment	Akinwumi et al. (2018)
Resveratrol (**1**), pterostilbene (**2**), and piceatannol (**5**)	Anti-inflammatory activity review of animal studies in mice	Anti-inflammatory activity of natural stilbenoids as a promising group of potential anti-inflammatory drugs	Dvorakova et al. (2017)
Oligostilbenes found in grapes and wine	Review work in mice	Antioxidant, anti-inflammatory, anticancer, antimicrobial, antiviral, cardioprotective, neuroprotective, and hepatoprotective activities of grape stilbenes	Georgiev et al. (2014)
Resveratrol (**1**), piceatannol (**5**), and pterostilbene (**2**)	Review study in mice	Effects of stilbenoids: as antitumor, antiplatelet, antioxidant, antimicrobial, anti-inflammatory, analgesic, anesthetic, and a behavior of membrane-interactive drugs	Tsuchiya (2015)
Resveratrol (**1**)	Animal study in mice and rats	Resveratrol and other stilbenes: potential inhibitory effect on transient receptor potential (TRP) channels and modulation of TRP channel activity	Yu et al. (2013)
Resveratrol (**1**), piceatannol (**5**), and *cis*-piceid (**27**) astringin, resveratroloside, *cis*-resveratroloside	Animal organ study in mice	Stilbenoids: potential inhibition of cyclooxygenases and preneoplastic lesions	Waffo-Téguo et al. (2001)

In a previous study involving animal organs, Waffo-Téguo et al. (2001) indicated that resveratrol (**1**) directly inhibits the activity of COX-2. The authors investigated the inhibitory effects of grape phenols on carcinogen-induced precancerous lesions in mouse mammary grand organ cultures. In this study, 12 phenols isolated from grape plant cell cultures were used to evaluate the potential of inhibition of cyclooxygenases and preneoplastic lesion formation in carcinogen-treated mouse mammary glands in organ culture. This report showed the potential cancer-chemo-preventive activity of astringin (**11**), a plant stilbenoid found in wine. Astringin (**11**) and its aglycone piceatannol (**5**) revealed activity in the mouse mammary gland

organ culture assay but displayed no activity in COX-1 and COX-2 assays. Resveratrol (**1**) presented bioactivity in the same bioassays indicating that astringin (**11**) and piceatannol (**5**) may act as potential cancer-chemopreventive agents using a different mechanism from that of resveratrol (**1**) (Waffo-Téguo et al. 2001).

Yu et al. (2013) investigated whether resveratrol (**1**) and other stilbenoids could modulate TRP (transient receptor potential) channels in sensory neurons *in vitro* and had analgesic effects *in vivo*, using whole-cell patch-clamp techniques and behavioral analysis. This study showed that resveratrol (**1**) and other stilbenoids have a potential inhibitory effect on TRP channels, exerting its activity in different ways. TRP is a family of ion channels that are activated by physical (temperature and mechanical force) and chemical stimuli. Sensory TRP channels are sensitized by pro-inflammatory agents and mediate heightened pain sensitivity.

Studies on obesity and diabetes and using animal models have concluded that, in addition to the known anti-inflammatory and antioxidant activities, grape seed extract prevents metabolic syndrome, type 2 diabetes, and obesity (by modulating the metabolic endotoxemia and by improving the gut barrier integrity; Georgiev et al. 2014).

In 2017, Dvorakova and Landa reviewed the anti-inflammatory activity of resveratrol (**1**), pterostilbene (**2**), and piceatannol (**5**). Resveratrol (**1**) was described to inhibit the expression of cyclooxygenase-2 (COX-2) *in vivo* models using rats. Its analgesic properties against pain (acute or chronic) could be assessed through alterations of the expression of serum tumor necrosis factor-alpha and whole brain nitric oxide in the diabetic rat model, reduction of expression of COX-2 in inflammatory pain model, or inhibition of cyclin-dependent kinase 5 activity in primary afferent neurons.

Recently, Akinwumi et al. (2018) reviewed the biological properties of stilbenoids such as resveratrol (**1**), pterostilbene (**2**), oxyresveratrol (**4**), piceatannol (**5**), and *cis*-piceid (**10**). The biological activities covered were from cardioprotection, neuroprotection, antidiabetic properties, depigmentation, and anti-inflammation to cancer prevention and treatment. The properties described are thought to be mediated by different universal signaling pathways. The hypolipidemic effect of the dietary resveratrol (**1**) was studied in hepatoma-bearing rats where the antitumor growth and anti-metastasis effects were also determined. In addition, resveratrol (**1**) was studied on isolated rat hearts from ischemia reperfusion injury, and its potential cardioprotective effects were attributed to its peroxyl radical scavenging activity (Akinwumi et al. 2018).

20.5 Human Benefits

The potential biological activities of wine stilbenes have been the subject of several *in vitro* and *in vivo* studies. However, the link between these preclinical studies and the beneficial effects in humans is not totally clarified yet. At the time of this review, there were 152 trials registered in ClinicalTrials.gov dealing with resveratrol (**1**). A recent review covered some of the latest clinical trials using this stilbene (Ramírez-Garza et al. 2018). However, only a few studies deal with the effects of

resveratrol or other stilbenes from grapes or wine. Herein, examples of relevant clinical trials using stilbenes and grape extracts are reviewed (Table 2).

The first clinical trial dealing with the potential beneficial effects of grape powder in patients with colon cancer was published in 2009 by Nguyen et al. (registration number NCT00256334). This pilot trial was performed as a four-dose cohorts study. Eight patients, preselected due to colon cancer diagnosis, were randomly assigned to each cohort, being treated orally every day until surgery (14 days). Thus, two patients ingested 120 g/day of grape powder (0.114 mg of resveratrol); three patients ingested 80 g/day of grape powder (0.073 mg of resveratrol); one patient had 80 mg/day of plant-derived resveratrol tablets (15.5 mg of resveratrol); and two patients ingested 20 mg/day of plant-derived resveratrol tablets (3.9 mg of resveratrol). At the times of diagnostic colonoscopy and surgery, cancer and normal colonic mucosa tissues were obtained. These samples were evaluated by Wnt (wingless/integrated) pathway-specific microarray and quantitative real-time polymerase chain reaction (qRT-PCR), comparing pre- and postexposure to resveratrol or grape powder. The Wnt signaling pathway activates mutations essential for the development of colon cancer. On the cancerous tissues, the treatment with resveratrol or grape powder had no change in the composite Wnt target gene expression. However, on normal colonic mucosa, the treatment with 80 g/day of grape powder inhibited the expression of cyclinD1 and axinII (Wnt target genes). This had the lowest resveratrol content of all the cohorts but also had many other bioactive components (other polyphenols) not present in the plant-derived resveratrol capsules. This clinical trial presented some limitations such as a small sample size and no control of the dietary intake of the patients nor control of medication that could affect Wnt pathway. But despite the limitations, the authors suggest that low doses of resveratrol or consumption of grapes may have a beneficial role in colon cancer prevention.

Besides cancer chemopreventive activity, consumption of wine and grapes has often been associated with benefits for cardiovascular system. In this regard, a clinical trial was designed to investigate the effects of a dietary resveratrol-rich grape supplement in primary and secondary prevention of cardiovascular disease (registration number: NCT01449110).

For primary prevention of cardiovascular disease studies, 75 patients on statin treatment and at high risk of cardiovascular disease (diabetes mellitus or hypercholesterolemia plus arterial hypertension, active tobacco smoking, or overweight/obesity) took part of this trial. The subjects were randomly distributed through the three parallel arms (triple-blinded and placebo controlled): grape extract containing 8 mg of resveratrol and other resveratrol derivatives piceid (**10**) and viniferins in trace amounts (group A; $n = 25$), a grape extract with a similar polyphenolic content but lacking resveratrol (group B; $n = 25$), and placebo (group C; maltodextrin; $n = 25$). The treatments were encapsulated, and the patients consumed 1 capsule/day for the first 6 months and 2 capsules/day for the next 6 months to assess possible dose-response effects. Therefore, to evaluate the effects on the atherogenic markers, the authors only used data after the first 6 months of treatment (Tomé-Carneiro et al. 2012a). Group A showed significant decreases in the low-density lipoprotein cholesterol (LDLc; −4.5%), apolipoprotein B (ApoB; −9.8%), oxidized LDL (LDLox;

Table 2 Summary of clinical trials using stilbenes and grape extracts

Condition	Study design	Dosages	Biomarkers	Effects	References
Colon cancer	Randomized four-dose cohorts ($n = 8$) No placebo Duration 14 days	120 g/day of grape powder ($n = 2$) 80 g/day of grape powder ($n = 3$) 80 mg/day of plant-derived resveratrol tablets ($n = 1$) 20 mg/day of plant-derived resveratrol tablets ($n = 2$)	Wnt pathway: inhibition of expression of cyclinD1 and axinII	Beneficial for prevention of colon cancer	Nguyen et al. (2009)
Cardiovascular disease(CVD) primary prevention	Randomized three parallel arms, placebo controlled ($n = 75$) Duration 12 months	1 capsule/day for 6 months +2 capsules/day for the next 6 months Group A: grape extract with 8 mg of resveratrol ($n = 25$) Group B: grape extract without resveratrol ($n = 25$) Placebo ($n = 25$)	Atherogenic markers: reduced levels of ApoB, LDLox (group A) Inflammatory and fibrinolytic markers: reduced levels of TNFα, hsCRP, and PAI1 and increased IL-10 (group A)	Beneficial for prevention of CVD on patients with statin medication	Tomé-Carneiro et al. (2012a, b)

Cardiovascular disease secondary prevention	Randomized three parallel arms, placebo controlled ($n = 75$) Duration 12 months	1 capsule/day for 6 months +2 capsules/day for the next 6 months Group A: grape extract with 8 mg of resveratrol ($n = 25$) Group B: grape extract without resveratrol ($n = 25$) Placebo ($n = 25$)	Inflammatory markers in PBMCs: increased levels of adiponectin and downregulation of Kruppel-like factor 2, NF-$_K$B, activator protein-1, c-Jun, activating transcription factor 2, and CREB-binding protein (group A)	Beneficial	Tomé-Carneiro et al. (2013a)
Diabetes mellitus type 2	Randomized three parallel arms, placebo controlled ($n = 35$ male type 2 diabetic and hypertensive medicated patients) Duration 12 months	Same as Tomé-Carneiro et al. (2013a)	Inflammatory markers: alteration of miR-21, miR-181b, miR-663, miR-30c2, miR-155, and miR-34a and reduction of CCL3, IL-1, and TNF-α (group A)	Beneficial	Tomé-Carneiro et al. (2013b)
Cardiovascular	Randomized 2 × 2 block design ($n = 80$, patients with hypercholesterolemia with or without cholesterol medication), placebo controlled. Duration 6–8 weeks	Twice a day Group A:50 mg pterostilbene Group B:125 mg pterostilbene Group C:50 mg pterostilbene +100 mg grape extract Placebo	Increase of LDL levels (groups A and B) Reduced blood pressure (group B)	Beneficial for hypertensive population	Riche et al. (2013)

−20%), and LDLox/ApoB (−12.5%). On the contrary, no significant changes were observed for both groups B and C. In conclusion, the intake of one capsule/day of resveratrol-rich grape supplement significantly reduced LDLox and ApoB in patients undergoing primary prevention of cardiovascular disease with statin medication (Tomé-Carneiro et al. 2012a). Furthermore, for the evaluation of inflammatory and fibrinolytic markers, Tomé-Carneiro et al. (2012b) measured serum levels of interleukin (IL)-6, IL-10, IL-18, tumor necrosis factor-α (TNFα), soluble intercellular adhesion molecule-1 (sICAM1), high-sensitivity C-reactive protein (hsCRP), adiponectin, and plasminogen activator inhibitor type 1 (PAI1). After 1-year treatment, the key findings of this study were that the resveratrol-rich grape supplement (group A) significantly decreased the inflammation-related markers hsCRP (−26%), TNFα (−19.8%), PAI1 (−16.8%), and IL-6/IL-10 ratio (−24%) and increased anti-inflammatory IL-10 (19.8%). No significant effects were observed for the placebo and grape extract without resveratrol groups. The authors concluded that 1-year consumption of grape supplement enriched in resveratrol improved the inflammatory and fibrinolytic status in patients who were on statins for prevention of cardiovascular artery disease (Tomé-Carneiro et al. 2012b).

For the secondary prevention of cardiovascular disease studies, 75 patients who coronary syndrome, cerebrovascular accident or peripheric arteriopathy event occurred at least 6 months or more, before the recruitment took part of this trial, which followed the same design as described above, using the same treatment groups and duration (Tomé-Carneiro et al. 2013a). After 1-year treatment, the transcriptional profiling of inflammatory genes in peripheral blood mononuclear cells (PBMCs) was explored using microarrays and functional gene expression analysis. The main outcomes were observed for the group treated with resveratrol-containing grape supplement (group A). The increased levels of adiponectin levels (10%) prevented the increase of PAI1 levels, and downregulation of pro-inflammatory transcription factors (Kruppel-like factor 2, NF-$_{\kappa}$B, activator protein-1, c-Jun, activating transcription factor 2, and CREB-binding protein) was found to be modulated in PBMCs. The non-high-density lipoprotein cholesterol load was reduced in both groups A and B. In contrast, the placebo group showed significant decrease of the levels of adiponectin and IL-10 and increase of PAI1. The authors concluded the presence of resveratrol in the grape supplement was essential for the observed benefic effects and pointed toward further research on this nutraceutical as a possible safe coadjuvant food supplement in the follow-up of coronary artery disease patients (Tomé-Carneiro et al. 2013a).

These human studies have evidenced the cardioprotective benefits of resveratrol and grape extracts through the amelioration of inflammatory and atherogenic markers. Nevertheless, many of these biochemical markers are also involved in the development of type 2 diabetes mellitus (T2DM). Therefore, Tomé-Carneiro et al. (2013b) further investigated the molecular changes associated to the regular intake of the resveratrol-containing grape extract in PBMCs isolated from

hypertensive patients with T2DM. A subset of 35 male type 2 diabetic and hypertensive medicated patients, which took part in the larger clinical trial previously described (Tomé-Carneiro et al. 2013a), were analyzed in this study. The authors evaluated the changes in genes and microRNAs involved in the inflammatory response. The supplementation of these patients with resveratrol-rich grape extract during 1 year highly correlated with the alteration of a group of miRNAs involved in the regulation of the inflammatory response (miR-21, miR-181b, miR-663, miR-30c2, miR-155, and miR-34a) as well as with the reduction of the expression of pro-inflammatory cytokines (CCL3, IL-1, and TNF-α) and increase of the transcriptional repressor LRRFIP-1 (leucine-rich repeat flightless-interacting protein). Although the authors conclude that long-term supplementation with a grape extract containing resveratrol has beneficial immunomodulatory effects in circulating immune cells of T2DM hypertensive medicated patients, they do not discard the hypothesis of the combined action of resveratrol with other phenolic compounds present in the grape extract or with some of the specific medication administered to these patients (Tomé-Carneiro et al. 2013b).

On the subject of benefic cardiovascular effects of stilbenes, another clinical trial concerning the activity of pterostilbene (**2**) on metabolic parameters was conducted (registration number, NCT01267227). Eighty subjects with hypercholesterolemia (total cholesterol $\geq$200 mg/dL and/or baseline low-density lipoprotein cholesterol $\geq$100 mg/dL) were randomized in a 2 $\times$ 2 block design for presence of cholesterol medication into one of four groups: 50 mg pterostilbene, 125 mg pterostilbene, 50 mg pterostilbene plus 100 mg grape extract, and placebo. The treatments were taken orally, twice a day for 6–8 weeks. The endpoints of this study were high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, blood pressure, and weight. In both groups treated with pterostilbene (**2**), there was an increase of LDL levels (17.1 mg/dL). This effect was not observed for the combination of pterostilbene (**2**) and grape extract. The presence of a baseline cholesterol medication appeared to attenuate LDL effects. Regarding the triglyceride levels, no significant results were observed for any group. Neither pterostilbene significantly affected HDL levels. Nevertheless, both systolic (−7.8 mmHg) and diastolic blood pressure (−7.3 mmHg) were reduced with 125 mg dose of pterostilbene. In conclusion, pterostilbene was able to increase LDL levels and reduce blood pressure when used in doses of 250 mg/day. The authors call the attention for future studies to evaluate high-dose pterostilbene (**2**) with grape extract in a hypertensive population (Riche et al. 2014). This clinical trial was also used to evaluate the effects on the safety of long-term pterostilbene administration in humans (Riche et al. 2013). Since some of the patients that took part on the clinical trial were taking statins, it was important to evaluate if there were any possible drug-drug interactions. It was concluded that pterostilbene did not demonstrate any biochemical hepatic adverse drug reactions nor had a direct effect of on measures of renal or glucose markers. In conclusion, pterostilbene was considered generally safe for use in humans up to 250 mg/day (Riche et al. 2013).

20.6 Application in Food: Wine Derivatives as Dietary Supplements and Related Products

Due to the health benefits attributed to polyphenolic compounds present in wine, during the last years, other options have been investigated. These include non-alcoholic wines, grape juices, powders, extracts, and other products obtained from grapevine, which show the same biological benefits but are devoid of the alcoholic content (Georgiev et al. 2016).

Regarding nonalcoholic wines, they can be an option for some social groups (as young adolescents, nondrinkers, drivers, etc.) who normally do not consume wine because of their content in alcohol. However, due to wine dealcoholization techniques, the quality, and, more importantly, the sensory characteristics are modified or even reduced. To overcome this setback, different techniques have been investigated in order to protect the organoleptic properties of nonalcoholic wines, for example, the membrane contactor and distillation under vacuum methods (Motta et al. 2017).

Without alcoholic content, powders obtained by freeze-dried wine and encapsulated in a maltodextrin matrix are being used as a source of wine polyphenols that can be added to other foods. This new product showed to have 3.7-fold more polyphenols than the same amount of red wine (Rocha-Parra et al. 2018).

A rich phenolic content powder can be also obtained from the pomace, the grape biomass discarded after the vinification process. A method to produce the dry pomace extract without using organic solvents was recently described. This extraction, based on aqueous cyclodextrins, showed to be an effective process to recovering phenolic compounds from food-derived products (Georgiev et al. 2016). It is known that pomaces obtained from red wines have higher content of phenolic and other natural compounds with antioxidant activity. Recently, Beres et al. (2017) reviewed the main applications of this powder grape pomace extract not only in food but also in pharmaceutical and cosmetic industries (Fig. 10).

By using a sieve, it is possible to separate the grape seeds from the pomace. Grape seeds have become increasingly popular on markets in different countries, such as the United States (Yamakoshi et al. 2002). From the seeds, it is possible to produce grape seed powder or grape seed oil or even dry grape seed extracts (Georgiev et al. 2016). These products are important due to their high polyphenolic content ranging from 60% to 70% of total extractable compounds and attract the interest of the pharmaceutical, cosmetic, and food industry as a cost-effective source of natural antioxidant compounds (Fig. 10). In market, it is possible to buy MegaNatural®-Gold Grape Seed Extract (Polyphenolics, USA) and Citricidal® grapefruit seed extract (NutriBiotic, USA) (Teixeira et al. 2014).

From the pomace, it is also possible to separate the grape skins by vibrating sieves. The powder obtained by prior dried and ground of the skins can be used as a nutraceutical or as a source to obtained pure antioxidant compounds, as the stilbenoid compound resveratrol (Georgiev et al. 2016). The pomace is also the origin of the so-called antioxidant dietary fibers. These soluble fibers are obtained by extraction of grape pomace using different techniques (Beres et al. 2017). Regarding their application (Fig. 10), some studies have shown the use of grape pomace and

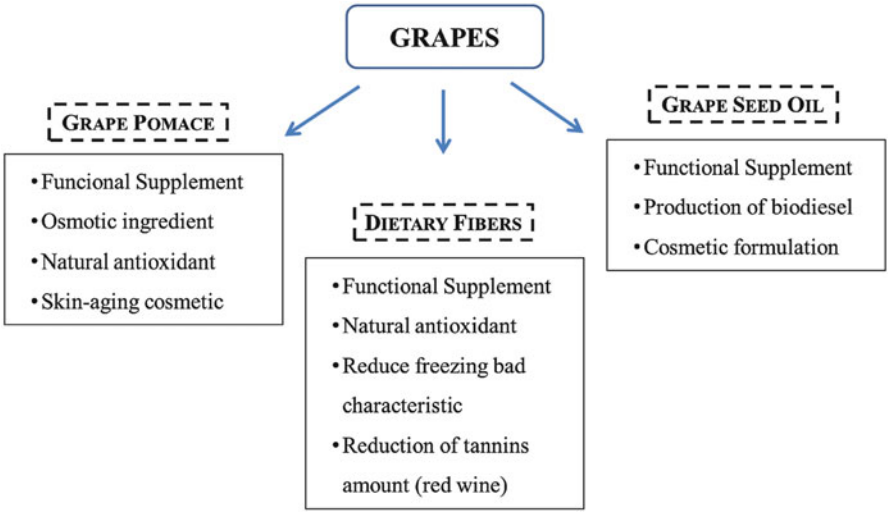

Fig. 10 Selected applications of grape derivatives

seed flours in different food products such as popsicles, cereal bars, biscuits and cookies, and muffins, in order to improve their content in antioxidant compounds (Beres et al. 2017). In order to prevent alterations such as flavor changes, color, texture, and lipid oxidation during freezing storage of some food products, for example, association of codfish or seafood with the extracted fibers obtained from pomace is being studied (Zhu et al. 2015; Beres et al. 2017). Moreover, studies in chicken breast hamburger are also being carried on (Beres et al. 2017).

20.7 Safety: Toxicity and Side Effects

During the last years, a high interest in natural product consumption and in particular resveratrol and related stilbenoids has been highlighted. The toxicity of resveratrol (**1**) has been exhaustively studied not only in animal models but also in humans. Regarding animal studies, the acute, subchronic, and chronic toxicity of resveratrol, synthesized by DSM Nutritional Products Ltd., was highly studied in multiple *in vitro* and *in vivo* animal assays (Williams et al. 2009). It revealed to be well tolerated and nontoxic, being a NOAEL (no-observed-adverse-effect level) for resveratrol defined as 0.75 g/kg bw/day dose (Edwards et al. 2011). In relation to its safety in humans, several studies have shown that at low doses, resveratrol is well tolerated; however more information is needed especially regarding its long-term use (Akinwumi et al. 2018). An important data is the acceptable daily intake (ADI) in humans for resveratrol, which was calculated by using data from animal toxicity studies and a standard default safety factor of 100 which gives an ADI of 0.45 g/day for a 60 kg individual (Edwards et al. 2011).

In a recent review (Wahab et al. 2017), it was reported that there are a small number of dose-independent adverse effects such as nephrotoxicity and gastrointestinal problems associated with administration of resveratrol in humans. It is highlighted that 0.45 g/day of resveratrol is a safe dose for a 60 kg person; however, its supplementation in higher doses could be slight toxic (Wahab et al. 2017). In fact, using daily doses of 2.5 g or higher amount of resveratrol, some minor side effects may occur, for example, nausea, vomiting, diarrhea, and liver dysfunction in patients with nonalcoholic fatty liver disease (Salehi et al. 2018). In another study, it is pointed that resveratrol was found to be safe and well tolerated and without any other new side effect when used in a long-term experiment or in doses up to 5 g/day (Ramírez-Garza et al. 2018). It is important to note that the results mentioned above were obtained in healthy population; therefore, caution must be taken concerning the results obtained in people that have some pathology (Salehi et al. 2018). Some of the last clinical trials using resveratrol are summarized in a recent review (Ramírez-Garza et al. 2018); however, further investigation is needed to evaluate the safety of resveratrol for long-term treatment.

It should be pointed that some adverse effects were also found for resveratrol. In fact, some studies have shown that resveratrol may act as a pro-oxidizing agent, contrarily to its widely known chemopreventive and antioxidant properties (Salehi et al. 2018).

It is extremely important to pay attention to possible interactions between resveratrol and other stilbenoid derivatives and conventional medicines. In fact, resveratrol may interact with several medical drugs (Salehi et al. 2018). In this regard, it was observed that high doses of resveratrol (1000 mg/day or above) inhibit cytochrome P450 isoenzymes such as CYP3A4, CYP2C9, and CYP2D6 and can induce CYP1A2, being responsible for a number of drug interactions, by changing drug clearance and consequently their bioavailability and toxicity (Wahab et al. 2017; Salehi et al. 2018). The interaction between resveratrol and anticoagulant, antiplatelet, or even nonsteroidal anti-inflammatory drugs could be a possibility, especially due to an intensification of both bruising and bleeding risk, associated to the reduction of human platelet aggregation activity showed by resveratrol when tested in *in vitro* assays (Salehi et al. 2018).

A recent review conducted by Zha (2018) showed also interaction between resveratrol and transporter proteins, especially with ABC transporter membrane proteins. In fact, it was reported that this natural compound could inhibit P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and organic anion transporter 1/3 (OAT1/OAT3) and consequently could enhance the exposure of patients to some anticancer drugs.

It is also important to emphasize the interaction of red wine polyphenols with human microbiota. A study conducted by Queipo-Ortuño et al. (2012) indicated that consumption of red wine significantly increase the growth of some important microbiota bacteria identities, namely, *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Bacteroides uniformis*, *Eggerthella lenta*, and *Blautia coccoides* suggesting its possible prebiotic benefit.

20.8 Marketed Products

Wine is the most popular and widely discussed nutritional grape product with proven beneficial health effects on human body, when moderately consumed. However, it is important to highlight the different content of resveratrol/stilbenoids among different wines. On the other hand, it is known that the effective concentration of resveratrol in order to achieve the claimed biological activities may be higher than that obtained from wine drinking or food consumption. These reasons led to the constant search of new food sources and emphasized the potential of nutritional stilbenoid supplements (Navarro et al. 2018). Nowadays, the number of new food supplements containing pure resveratrol or this in a mixture of compounds is growing. However, mainly due to inadequate industry quality control, the majority of these supplements do not have the claimed concentration of resveratrol. This issue is highlighted by the work of Rossi et al. (2012). Resveratrol is also used by cosmetic industry. One promising and famous example is the cosmetic trademark Caudalie®, whose products contain as main ingredients resveratrol and other grapeseed polyphenols and stilbenoids. Its first patent is dated from 1999 and was related with compositions which are essentiality resveratrol esters, in the form of monomers and/or oligomers (Vercauteren et al. 1999). However, the search for new derivatives and methods to improve its cosmetic products is intense and constant as highlighted by a recent patent presented by the same industry. This patent is related with a new process for stimulating hyaluronic acid synthesis using resveratrol monomers and/or oligomers (Thomas et al. 2016). As showed above, the selection of the source of resveratrol is very important in order to obtain high amount of resveratrol and related stilbenoids with a realistic price. From the different strategies known, namely, the chemical synthesis and the use of by-products derived from tropical foods (mango pulp or even the guava) or from plants with little economical values or even from by-products of harvesting or wine production, the use of biotechnology and genetic engineering is increasingly attracting attention in the last years (Navarro et al. 2018). In fact, there are different engineering techniques that have been studied, namely, the production of resveratrol and related compounds, using microorganisms and enzymes. Kuo et al. (2017) described a successful approach to have mass production of resveratrol by using a small portion of extract of *Polygonum cuspidatum* and the wine yeast *Dekkera bruxellensis*. In this way, a repeated fed-batch fermentation process was scaled up to 1200 L in order to produce 35 mg of resveratrol per hour, liter, and round (Kuo et al. 2017). To produce higher quantity of resveratrol and other natural compounds, the genetic engineering, namely, transgenic organisms, is being used with very good results. A recent review summarized current progresses made in resveratrol biosynthesis, using bacterial hosts (Braga et al. 2018).

Regarding biotechnological approaches, plant cell biotechnology of grapes and mainly grape cell suspensions are seen as promising and alternative methods for producing bioactive nutraceuticals. In fact, the growth of grape cells in bioreactors for their production displays advantages when compared to the conventional breeding. In order to increase yields of these phytochemicals, several optimization

strategies have been used, as exemplified for the non-resveratrol-producing grapevine cell suspension of a grape hybrid, which produced resveratrol in the presence of methyl jasmonate. When scaling up the culture to a laboratory bioreactor, it achieved a yield of 230 mg/L (Georgiev et al. 2014).

20.9 Patents

Resveratrol is widely known as possessing a highly variety of biological benefits, including antitumor, antioxidant, anti-inflammatory, cardioprotective, neuroprotective, and other activities. Due to all beneficial activities, the number of food supplements containing resveratrol increases every day, and their study, as medicines, is a reality. However, their therapeutic use has been hindered by a number of factors that need to be solved. On one hand, until now, it is not known the real cellular or molecular targets of resveratrol. On the other hand, its low bioavailability and possible oxidative degradation during preparation for biological applications hamper the finding of a therapeutic dose that can benefit human health. These main reasons led to the development of different resveratrol derivatives with the objective of improving the therapeutic potential of resveratrol-based compounds (Li et al. 2016).

In a recent review, a total of 29 patents and over 200 resveratrol derivatives were reported. These derivatives were studied mainly to cancer, cardiac diseases, metabolic disorders, and neurologic diseases. The cited review also refers some studies regarding the application of resveratrol derivative patents in nutraceutical compositions and cosmetics (Li et al. 2016). In another review of patents, made from 2009 to 2012, these two classes of applications have been also discussed together with findings in the main therapeutic areas referred above (Pezzuto et al. 2013).

In a search of new patents from the last year, the same areas of research are pointed. As an example, a German patent describes the process for producing a skin care formulation containing dimers of resveratrol. This formulation could be used to topical application or pharmaceutical preparation of cosmetic skin care (Merryvital 2018).

20.10 Conclusions and Perspectives

Summarizing, stilbenoids are plant-derived polyphenols with several biological activities, which have been attracting increasing interest due to their potential benefits for human health. They can be found as monomers or very complex oligomers resulting from oxidative coupling of monomeric stilbenes, whose structural variations include different substituent patterns in the aryl rings. In human diet, the principal sources of stilbenes are grapes and wine. Despite the beneficial evidences of stilbenes on human health, there is still some ambiguity between high bioactivity and low bioavailability, namely, for resveratrol, the most studied stilbene. Consequently, further studies are crucial for evaluating the bioavailability and

other pharmacokinetic parameters of stilbenes, thus providing evidence for their therapeutic importance in human health. Their potential biological activities have been the subject of several *in vitro* and *in vivo* studies. Despite the reduced number of registered clinical trials addressing the human benefits of stilbenes from wine or grapes, the ones reviewed herein point to an increasing evidence that resveratrol and pterostilbene exert beneficial cardioprotective effects in medicated patients. Nevertheless, further studies are needed to unveil the underlying mechanisms by which this may occur and to establish correlations between the promising effects observed in animal models.

Due to the health benefits attributed to stilbenes in human health, the number of food supplements, medicines, and cosmetics in the market is increasing every day. However, in order to increase the number of stilbenes in health-promoting products, there are still some issues that need to be solved, namely, the source of resveratrol and quality control in industry production.

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Chlorogenic, Caffeic, and Ferulic Acids and Their Derivatives in Foods

21

Perumal Manivel and Xiumin Chen

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Abstract

Chlorogenic (CGAs), caffeic (CA), and ferulic acids (FA) are ubiquitously exist in plant foods, especially in coffee which is the most consumed beverages with fascinating economic, botanical, and historical significances. In the recent 20 years, there was an increase of interest in the health benefits of coffee in which CGAs, CA, and FA are considered important contributors for the bioactivities. Numerous scientific evidences exhibit that these phenolic components render a number of diverse biological activities including antioxidant, antimicrobial, anti-inflammatory, antiviral, anti-carcinogenicity, and antiaging effects. They are used as functional component in foods and various commercial products. This chapter reviewed the bioactive constituents, bioavailability, and metabolism of CGAs, CA, and FA and their derivatives in food, especially in coffee. We also summarized the biological activities of these phenolic acids in animal and human studies along with their toxicity and side effects, application in food, marketing products, as well as related patents.

Keywords

Coffee · Green coffee bean · Chlorogenic acid · Caffeic acid · Ferulic acid · Bioavailability · Bioactivity

21.1 Introduction

Clinical and epidemiological investigations have led to basic research for unraveling the chemistry and mechanisms of action of dietary phytochemicals. These studies have established the relationship between food or diet and health status. Natural health products and functional foods encompass a wide range of food/ingredients, with a variety of bioactive molecules which accountable for their efficacy in disease prevention and health promotion. Chlorogenic (CGAs), caffeic (CA), and ferulic acids (FA) are a significant group of secondary plant metabolites and are of substantial interest due to their perceived advantageous effects on health.

Plant origin foods such as coffee, yerba mate tea, fruits, and vegetables are rich in CGAs, CA, and FA (Table 1), among which coffee, especially green coffee bean, is one of the richest sources for CGAs (Saeed et al. 2019; Bagchi et al. 2016). Green

Table 1 Content of CGA, CA and FA in various sources of food

Sources	Foods	Content (mg/100g fresh weight)		
		CGA	CA	FA
Beverages	Coffee	8.6–11.0[a]	-	9.1–14.3
	Coffee leaves	2.4–3.9[d]	-	2.08–12.5[d]
	Green coffee beans	3400–14400[a]	33–141[a]	6–35[a]
	Roasted coffee beans	1500–3800[a]	-	-
	Tea beverage	0.2	-	-
	Tea shoots	559–674	-	-
	Yerba Mate	4580–8080[a]	0.023[a]	-
Fruits	Apple	1.93–119.5	-	0.27–0.85
	Apricot	3.0–16.5	-	-
	Banana	-	-	5.4
	Blackberries	7	1.38–3.64	2.99–3.51
	Black chokeberries	1.8[b]	-	-
	Blackcurrants	14	-	-
	Blueberries	0.5-2[b]	0–6.32	3.02–16.97
	Cranberries	-	0.38–15.6	0.8–8.8
	English apple cider	6-587 [c]	-	-
	Gooseberries	2-3	-	-
	Grapefruit	2.7–6.2	-	10.7–11.6
	Grape juice	10–430[c]	-	-
	Kiwi	11[c]	-	-
	Lemon	5.5–6.7	-	-
	Nectarines	2.3–27.7	-	-
	Orange	17–25	-	9.2–9.9
	Peaches	2.4–24.2	-	-
	Pear	6–59	-	-
	Plum	1.5–1.9 [b]	-	1.47
	Prunes	41.1–43.6	-	-
	Raspberries	2-3	-	-
	Strawberries	2-3	-	
	Sour cherries	0.6–5.8	-	-
	Sweet cherries	3.2–12.0	-	-
	Tomato	8.5	-	0.29–6
	Quince pulp	0.56–18.71	-	-
Vegetables	Artichoke heads	389[a] (cynarin)	-	-
	Asparagus	-	-	46.1
	Bamboo shoots	-	-	243.6
	Beet root	-	-	1.3–14.3
	Black carrot root	65.7[a]	-	-
	Burdock	-	-	7.3–19
	Broccoli	60[c]	-	4.1
	Brussels sprouts	3.7	-	-
	Cabbage	10.4	-	6.3–6.5

(continued)

Table 1 (continued)

Sources	Foods	Content (mg/100g fresh weight)		
		CGA	CA	FA
	Carrot	2–12	14	1.2–2.8
	Cauliflower	2	-	-
	Chicory root	10[a]	-	-
	Chinese cabbage	-	-	1.4
	Eggplant	-	-	7.3–3.5
	Kale	0.6–12	-	-
	Lettuce	5–12	4–55	0.19–1.4
	Potato	0.35–18.71	3.6	-
	Radish	2.4–5.0	-	4.6
	Pickled Redbeet	-	-	39
	Soyabean	-	-	12
	Sweet potato	4.6–13.6	0.3–2.2	-
Other Sources	Barley bran	5	-	-
	Rice	12[b]	-	24
	Spinach/frozen	-	-	7.4
	Sugar-beet pulp	-	-	800

[a]Per100 g dry weight; [b]g/kg weight; [c]mg/l; [d]% d.b.
Source: Adopted from Andres-Lacueva et al. 2010; Bagchi et al. 2016; Butiuk et al. 2016; Chen 2019; Gao et al. 1994; Hernandez et al. 1997; Marks et al. 2007; Mattila et al. 2005; Micard et al. 1997; Nakatani et al. 2000; Nishizawa et al. 1998; Risch and Herrmann 1988; Sakakibara et al. 2003; Saeed et al. 2019; Slimestad and Verheul 2009; Spanos and Wrolstad 1992; Zhao and Moghadasian 2008.

coffee bean contains 5–12 g/100 g CGAs (Sanlier et al. 2019), and the content of CGAs in roasted coffee depends on the degree of roasting and the type of beans. The loss of CGAs in roasted coffee beans ranges from 60% to 98% during the light or very dark roasting process. Daily intake of phenolic compounds ranges from less than 100 mg to over 2 g (Clifford 2004). In western countries, daily intake of coffee provides more than 1 g of CGAs, which is considered as the major contribution for the dietary phenolics (Del Rio et al. 2013). Except for coffee beans, coffee leaves, the by-product of coffee plants, have been made into tealike beverage which also contains abundant CGAs, CA, and FA. The content of these phenolic compounds depends on the species and cultivar of coffee plants, age of leaves, and the processing methods (Campa et al. 2012; Chen 2019; Chen et al. 2018b). *Coffea arabica* leaves contain higher (3.9% of dry leaves) CGA compared with that of *Coffea canephora* (robusta) leaves (2.4% of dry leaves) (Campa et al. 2012). Japanese-style-green tea-processed young coffee leaves retained greater CGAs than that of black tea-processed mature leaves (Chen et al. 2018b).

CA, the most common hydroxycinnamic acids, accounts for up to 70% of total hydroxycinnamic acids in fruits (Lafay and Gil-Izquierdo 2008). FA exists widely in cereal grains. FA is a commonly used food preservative that exhibited a wide range of biological applications such as antioxidant, immune-modulatory, growth-

enhancing, and antibacterial activities. They are generally esterified with quinic and tartaric acids or form conjugate with sugar. CGAs, CA, and FA are known to possess various bioactivities including antioxidant, anti-inflammatory, antimicrobial, anti-allergic, antidiabetic, antilipidemic, cardioprotective, neuroprotection, and hepatoprotective effects (Wianowska and Gil 2019; Espindola et al. 2019). Clinical studies also showed that consumption of CGA-, CA-, and FA-rich foods or administration of those pure phenolic acids has positive effects on Alzheimer's disease, Parkinson's disease, neurological behavior (infant hyperactivity), psychoactive responses, diabetes, gonad and liver function, cancer, and blood pressure (Dorea and Da Costa 2005). However, overdose of CGAs, CA, and FA also showed toxicity and side effects, such as causing hypotension, cardiovascular diseases, genotoxicity, and reproductivity problem (Butt and Sultan 2011).

In order to show efficacy in vivo, bioactive food components need to be bioavailable first. From a nutritional perspective, bioavailability is used to quantify the proportion of intake food that can be utilized by the human body and thus show efficacy. Bioavailability is a multistep process that involves releasing of bioactive components from the food matrix, absorption through the intestine, distribution to the circulation system and different organs, metabolism by phase I and II enzymes, and thus elimination out of body (Rein et al. 2013). Bioavailability is influenced by the bioaccessibility, composition of food matrix, structure and polarity of bioactive components, the transporters and enzymes, and gut microbiota. In food, the majority of phenolic compounds exist as conjugate form with other macromolecules, instead of free form. In the human body, after metabolization, phenolic compounds turn into different metabolites that possess different bioactivities compared with their parent compounds. Therefore, understanding the bioavailability of bioactive compounds from different food matrices and the efficacy of metabolites are very crucial for the utilization of food bioactivities.

The aim of this chapter is to summarize the bioactive constituents, bioavailability, and metabolism of CGAs, CA, and FA with their derivatives in food with an especial focus on coffee, as well as on their unique biological activities on animal and human, the toxicity and side effects, the marked product, and patents that related to those compounds.

21.2 Bioactive Constituents

Chlorogenic acid (CGA) was discovered in 1837 and was introduced in 1846 to describe a rich component in the green coffee bean, which was first recognized as 3-O-caffeoylquinic acid (3-CQA) and later as 5-O-caffeoylquinic acid (5-CQA) by the IUPAC. The term "chlorogenic acids" (CGAs) issued to described a group of esters that are formed between quinic acid and hydroxycinnamic acids (ferulic, caffeic, p-coumaric acids, and sinapic acid). Therefore, in the present chapter, CGA is used to represent 5-CQA, and CGAs is used to denote chlorogenic acids and their

derivatives. Currently, over 300 major and minor CGAs have been described in coffee and other plant materials (Farah and Lima 2019). These compounds include caffeoylquinic acids (CQAs: 3-CQA, 4-CQA, and 5-CQA), dicaffeoylquinic acids (diCQAs: 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA), feruloylquinic acids (FQAs: 3-FQA, 4-FQA, and 5-FQA), diferuloylquinic acids (diFQAs: 3,4-diFQA, 3,5-diFQA, and 4,5-diFQA), *p*-coumaroylquinic acid (*p*CoQAs: 3-*p*CoQA, 4-*p*CoQA, and 5-*p*CoQA), di-*p*-coumaroylquinic (di*p*CoQAs: 3,4-di*p*CoQAs, 3,5-di*p*CoQAs, and 4,5-di*p*CoQAs), sinapoylquinic acids (SiQAs: 3-SiQA, 4-SiQA, and 5-SiQA), dimethoxycinnamoylquinic acids (DQAs: 3-DQA, 4-DQA, and 5-DQA), caffeoyl-feruloylquinic acids (3F-4CQA, 3C-4FQA, 3F-5CQA, 3C-5FQA, 4F-5CQA, and 4C-5FQA), trimethoxycinnamoylcaffeoyl- or feruloylquinic acids (4T-5CQA, 3T-5CQA, 3T-5FQA, 3T-4FQA, and 4T-5FQA), caffeoyl-dimethoxycinnamoylquinic acids (3D-4CQA, 3D-5CQA, and 4D-5CQA), feruloyldimethoxycinnamoylquinic acid (3D-4FQA, 3D-5FQA, and 4D-5FQA), sinapoyl-caffeoylquinic acids (3Si-5CQA, 3Si-4CQA, and 4Si-3CQA), sinapoyl-feruloylquinic acids (3Si-5FQA, 4Si-5FQA, and 4Si-3FQA), and related compounds. Majority of them that existed in coffee and coffee leaves belong to three subclasses: CQAs, di-CQAs, and FQAs (Fig. 1) (Chen 2019; Clifford et al. 2003).

The major dietary sources of CGAs are vegetables (e.g., eggplant, tomato, carrot, and sweet potato), fruits (e.g. apple, pears, cherry, blueberry, and strawberry), and beverage (e.g., coffee and tea) (Rohit and Rao 2013). Green coffee is the major source of CGAs in nature, the content is about 5–12 g/100 g (Farah and Donangelo 2006), and CGA content is affected by the species and cultivars. For example, CGA contents in green coffee beans of *C. canephora* and *C. arabica* are 5.17–14.4% and 3.40–7.24%, respectively (Narita and Inouye 2015). After roasting, CGA contents significantly decrease to 1.7–2.5 g/100 g and 1.0–4.3 g/100 g in the medium roasted *C. canephora* and *C. arabica* beans, respectively (Farah and Lima 2019). Among CGA derivatives, 5-CQA has been reported as the most predominant compound in coffee beans and leaves and most of the plants (Chen et al. 2018b; Chen 2019).

CA, the most abundant hydroxycinnamic acids, presents mainly in coffee beans, fruits, olives, carrots, potatoes, propolis, and olives. CA (3,4-dihydroxycinnamic acid) consists of phenylpropanoid (C6-C3) configuration with a 3,4-dihydroxylated aromatic ring attached to a -COOH through a trans-ethylene wire (Fig. 1). It exists in various forms such as monomers (glycosides, amides, organic acid, and sugar esters), dimmers, trimers, and flavonoid derivatives. Further, these compounds are found in the cell wall of vegetables in the bound form with proteins or polymers (Espindola et al. 2019).

Similar to CA, FA is also a hydroxycinnamic acid in which 3-OH is substituted with methoxy group (Fig. 1). FA is also an abundant phenolic acid in plants, e.g., 5 g/kg in wheat brain and 50 g/kg in corn kernel. In coffee, FA content is about 9.1–14.3 mg/100 g (Saeed et al. 2019). FA occurs as free form or covalently conjugated to polysaccharides, polyamines, hydroxyl fatty acids, lignin, and glycoproteins in the plant cell wall. In cereals, it is esterified to the arabinoxylans of the grain cell walls.

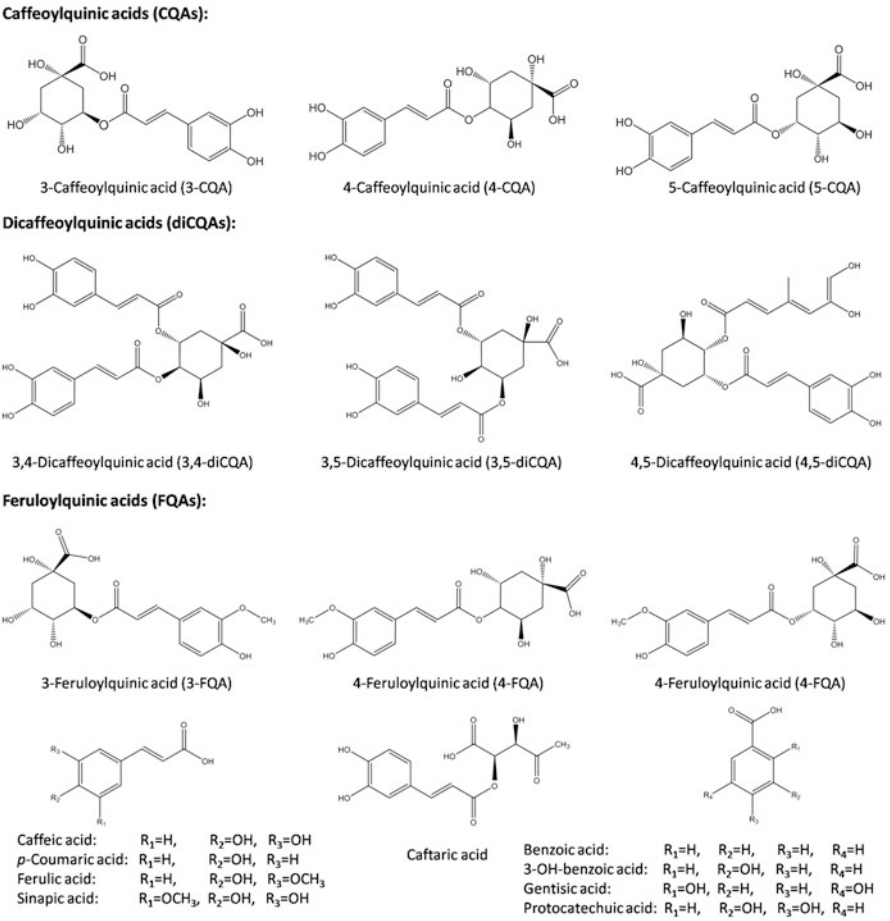

Fig. 1 Chemical structures of major CGAs, CA, and FA in coffee

21.3 Bioavailability and Metabolism

Food matrix is a very complex system that affects the bioaccessibility and bioavailability of phenolic compounds. CGAs, CA, and FA widely present in plants as conjugated forms instead of free forms. They exist as low-molecular-weight, water-soluble components in cytosol as well as lipid-soluble compounds in the waxes of the plant surface (Karakaya 2004). The conjugation results in less bioaccessibility as well as enhanced hydrophilicity and molecular weight, which cause reduced bioavailability. After oral injection, phenolic compounds undergo an intestinal transformation before absorption and entering the circulation toward the liver and other organs. The nonabsorbed fraction is enzymatically metabolized by gut microbiota to produce various metabolites.

21.3.1 Chlorogenic Acid

The bioavailability and metabolite of CGA have been better studied compared with other phenolic acids due to its abundance in foods, especially in coffee. Understanding of CGA metabolism in human body is essential to reveal the fate and efficacy of CGA in vivo. The studies of bioavailability and metabolism of CGA and CA started in the 1950s. Previously, it was thought that the bioavailability of CGAs is very poor. Less than 1% of oral intake CGA was absorbed by animal and human, and most of it was metabolized by gut microbiota. However, thanks to the improved detection techniques, nowadays, it is known that about one third of injected CGAs is absorbed by human gastrointestinal (GI) track (Farah and Lima 2019). The absorption of CGA starts at the upper session of GI track, the stomach, but mostly happens in the small intestine through passive diffusion (Monteiro et al. 2007). Monocarboxylic acid transporter and bilitranslocase are also considered involved in the absorption of CGA.

About 30% intake of CGA is absorbed in the small intestine, and the rest of it reaches the colon and utilized by gut microbiota (Stalmach et al. 2009). Colonic microflora hydrolyzed the unabsorbed CGA to CA and quinic acid in the colon. Further, it is dehydroxylated to form 3-hydroxyphenylpropionic and benzoic acid, and then the hippuric acid is formed when conjugated with glycine. As a consequence, half of the ingested CGA appears as urinary hippuric acid (Olthof et al. 2001). It was previously found that there were only $<1\%$ of CGAs ingested identified and quantified in murine or human plasma and urine due to digestion and metabolized process (Monteiro et al. 2007; Gonthier et al. 2006). Farah et al. (2008) identified the considerable amount of CGA, CQA, diCQA, and FQA compounds in human plasma after consumption of roasted and green coffee extracts. After absorption, CGA undergo phase II metabolism to form sulfate and glucuronide conjugates. The schematic representation of the main metabolic pathway of CGAs is shown in Fig. 2. It can be noticed that the major FA and CA metabolites like dihydro ferulic acid-4-O-sulfate, dihydro caffeic acid-3-O-sulfate, and caffeic acid-3-O-sulfate are found in the blood circulation of CGA metabolism (Marin et al. 2015; Naveed et al. 2018). Due to limited studies available on the biological properties of CGA, FQA, FQA, p-CoQA, CFQA (caffeoyl-feruloylquinic acid), and other minor CGA compounds, more studies are needed to establish the mechanisms involved in the metabolism and absorption of individual major/minor CGA compounds from coffee.

The bioavailability of CGAs was largely dependent on the food matrix and varied among individuals. It was reported that CGA absorption was not affected by milk or sugar that were added during coffee consumption. The transition of CGA in the upper part of the gut is very rapid in food-deprived rats or fasting humans when administrated with coffee or pure CGA; therefore, no CGA was detected in plasma or urine (Azuma et al. 2000). However, when volunteers consumed prune or coffee with breakfast, CGA appeared in urine (Ito et al. 2005).

In summary, the fate of CGA in vivo can be divided into three parts: a part of it was absorbed from the stomach intact; some of it is absorbed by small intestine

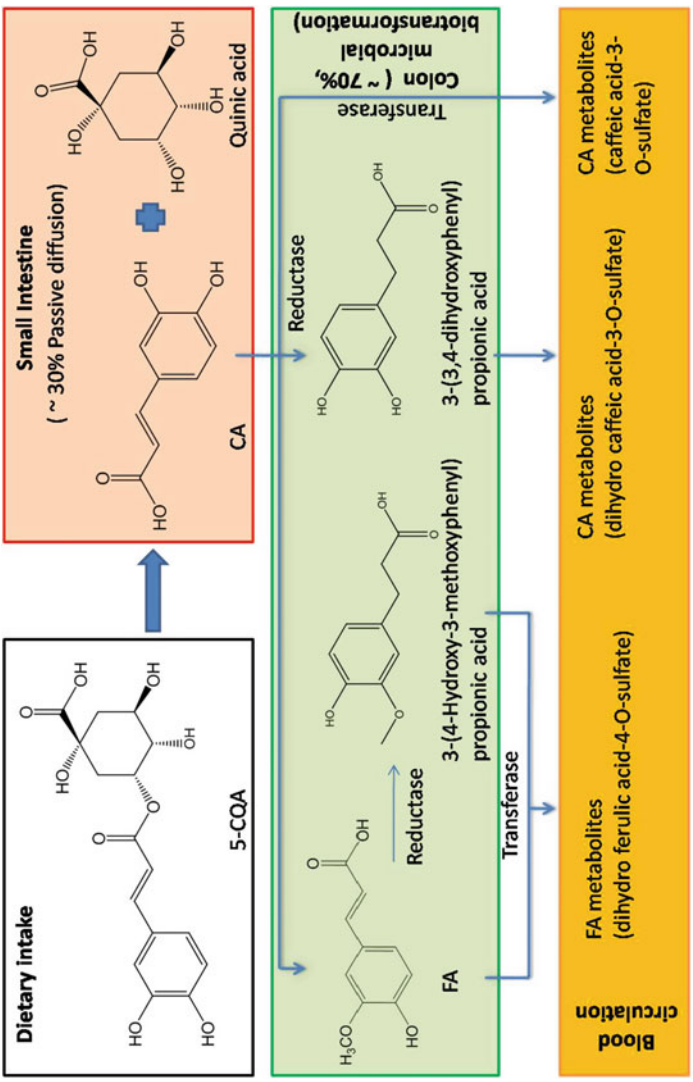

Fig. 2 Major metabolic pathway of 5-CQA and CA (Adapted from Naveed et al. 2018)

followed by hydrolyzed by the enterocytes before entering circulation; the last fraction of the not absorbed CGA reached caecum and metabolized by gut microbiota.

21.3.2 Caffeic Acid

When rats administrated CA through gastric intubation, CA, FA, and their sulfates or glucuronides were found in plasma 30 min after administration, and the metabolites reached maximum 2 h later (Azuma et al. 2000). CA was also found to be absorbed in the stomach (Konishi et al. 2006) and distributed in the portal vein and aorta 10 min after its administration (Konishi et al. 2005). About 19.1% of CA can be absorbed in rat small intestine, whereas 11% of the ingested dose was found in urine of ileostomy volunteers (Olthof et al. 2001). However, only 0.3% of CGA excreted in urine, indicating that esterification of CA with quinic acid markedly decreased the absorption of CGA. CA can also be metabolized by gut microbiota. The two key microbial metabolites of CA are 3-hydroxyphenylpropionic acid and benzoic acid, which are produced by the action of *Bifidobacterium lactis*, *Lactobacillus gasseri*, and *Escherichia coli* (Gonthier et al. 2006). Isoferulic acid has been found as a specific metabolite of dietary CA derivatives. It was revealed as a useful biomarker for the metabolism of CA derivatives from dietary sources (Rechner et al. 2001).

21.3.3 Ferulic Acid

The physiological significance of FA is affected by its bioavailability for absorption and subsequent interaction with target organs. It is found that after 24 min of oral administration, free FA reached highest concentration in plasma level (Zhao and Moghadasian 2008). The metabolic pathway of FA feruloyl monomer, oligomer, and polymer are summarized in Fig. 3. Feruloyl monomers get hydrolyzed to form free FA in the intestinal lumen or mucosa, and then FA-glucuronides are generated in the intestinal mucosa. In the liver, free FA and FA-glucuronides are metabolized into several metabolites including FA-sulfate, FA-glucuronide, and FA-sulfoglucuronide which is the FA diconjugate form with glucuronide and sulfate, feruloylglycine, dihydroferulic acid, m-hydroxyphenylpropionic acid, vanillic acid, and vanilloylglycine (Zhao et al. 2003; Zhao and Moghadasian 2008). Further, vanillin is changed into oxaloacetate and pyruvate by protocatechuate (PCA) 4,5-cleavage pathway. Finally, FA catabolism end products enter into the tricarboxylic acid cycle and generate energy in the biological system. In addition, it is also reported that the administration of FA in the intraperitoneal to rats excretes 3-hydroxyphenyl propionic acid as a major urinary metabolite (Zhao et al. 2003; Zhao and Moghadasian 2008).

The bioavailability of FA is greatly affected by food matrix as well. For example, FA was detected in plasma of volunteers shortly after consumption of beer and the urinary excretion reached maximum at 7 h after injection (Bourne et al. 2000). Only

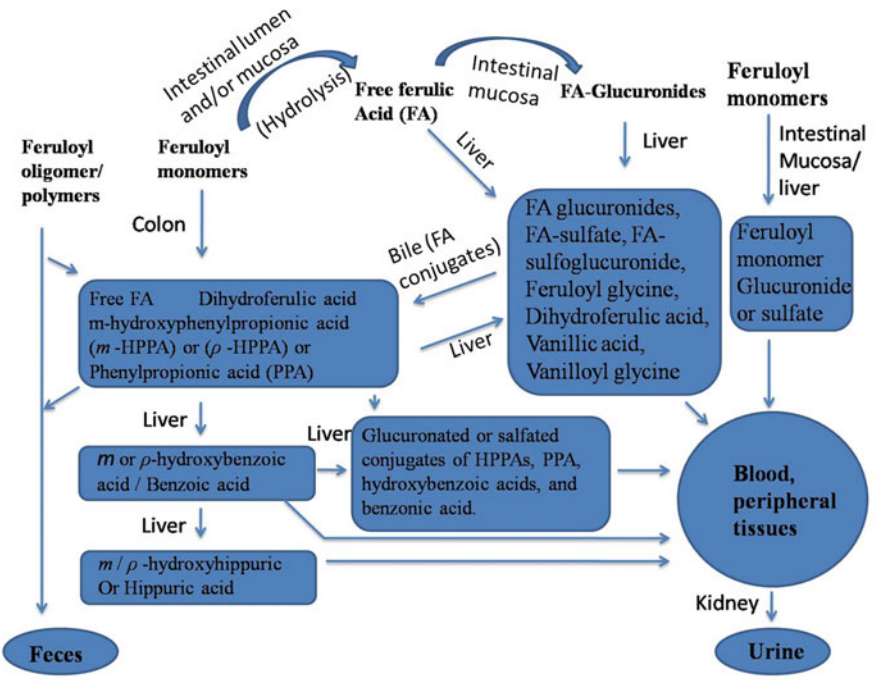

Fig. 3 Proposed possible metabolic pathways of dietary FA, reprinted with some modification from the ref. Zhao and Moghadasian 2008, copyright 2008, with permission from Elsevier

3% of ingested FA through cereals was detected in rat urine; however, the urinary exertion reached 50% of intake when free FA was administrated (Adam et al. 2002). This is because in the whole grain foods, FA is esterified with araboxylans and hemicelluloses, which resulted in the decreased bioavailability. Free FA was absorbed in the upper part of the gut, whereas the esterified FA metabolized in the caecum (Zhao et al. 2003). Moreover, the major microbial metabolites of FA are vanillin and 3-(4-hydroxyphenyl)-propionic acid (Andreasen et al. 2001). It is to be noticed that the bioavailability of FA varied in human and rats. For example, Yang et al. (2007) found the rapid absorption of FA upon oral administration, whereas FA has been reported to have very low oral bioavailability in humans because of its extensive metabolism.

A recent study compared the bioavailability of four hydroxycinnamic acids including CGA, CA, FA, and *p*-coumaric acid in non-fasted rats under physiological conditions. The injected CA, FA, and p-coumaric acid mainly excreted in the urine within 0–6 h as free form or glucuronide and/or sulfate conjugates, whereas CGA metabolites including CA, FA, *p*-coumaric acid and their conjugates were detected at 6–24 h and 24–48 h in the urine. The order of overall urinary excretion at 48 h was as followed: ferulic acid (73.2%) > caffeic acid (61.6%) > p-coumaric acid (54.1%) >> chlorogenic acid (4.9%). The percentages of the conjugates followed a

descending order as: 96% for ferulic acid >83% for caffeic acid >74% for chlorogenic acid >68% for p-coumaric acid. The results indicated that CGA was much poorly bioavailable and slower excretion compared with FA, CA, and p-coumaric acid due to the esterification of CA with quinic acid (Kishida and Matsumoto 2019).

21.4 Bioactivities (Animal Aspects)

CGA, CA, and FA are phenolic acids that are widely distributed in the human diet. Therefore, increasing research interest focuses on their enormous health benefits in human. There are numerous in vitro and animal studies related to the biological activities of CGA, whereas the clinical studies are relatively scant. Most of the human studies are related to coffee and much less used pure CGA, CA, and FA. Due to the lack of commercial availability of most minor CGAs derivatives, CGA is the most widely researched.

The possible mechanism of action and various beneficial activities of CGA are shown in Fig. 4. CGA reduces reactive oxygen species (ROS) and

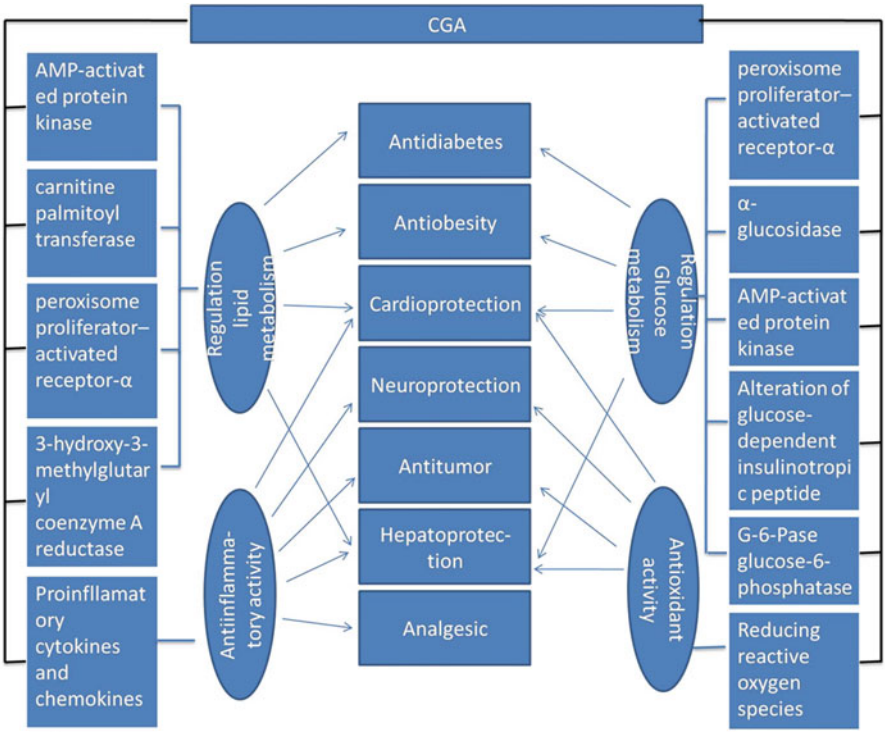

Fig. 4 Mechanisms of action of CGAs and its various health benefits (Adapted from Tajik et al. 2017)

proinflammatory cytokines or chemokines, and thereby it shows antioxidant and anti-inflammatory activity, respectively. Due to the antioxidant and anti-inflammatory activity of CGA, it has exhibited several promising applications including antidiabetes, anti-obesity, cardioprotection, neuroprotection, antitumor, hepatoprotection, and analgesic activities. CA and FA have also showed various biological applications. The recent animal studies on the bioactivities of CGA, CA, and FA and its derivatives are displayed in Table 2.

21.4.1 Antioxidant Activity

Oxidative stress is related to several chronic diseases, degenerative diseases, cancer, and aging. Antioxidants are valuable compounds that inhibit/reduce the effects activated by free radicals and oxidizing agents. Phenolic compounds are well-known antioxidants that show various health benefits. CGA is of special interest due to its unique properties and is able to fight oxidative stress by acting as a metal chelator, reducing lipid peroxidation, inhibiting NAD(P)H oxidase activity, and scavenging free radicals (Clifford 1999; Tajik et al. 2017). Animal studies indicated that the antioxidant and anti-inflammatory activities of CGA contribute to the health effects of coffee. Chen et al. (2018a) found administration of CGA to weaned pig reduced the oxidative stress, increased the intestinal absorption and digestion function, thus ameliorated weaning stress-induced diarrhea, and enhanced the growth performances of weaned pigs. Bao et al. (2018) proposed that dietary intervention rich in CGA has been used as an effective therapeutic agent for the treatment of diabetic nephropathy which is associated with the antioxidant effect of CGA. When diabetic rats fed with CGA, the lipid hydroperoxide was decreased, and the antioxidants such as reduced glutathione and vitamins C and E were increased (Karthikesan et al. 2010; Pari et al. 2010). In the methamphetamine-treated rats, CGA was able to reduce the oxidative stress by increasing liver superoxide dismutase and glutathione peroxidase activities and inhibiting lipid oxidation (Koriem and Soliman 2014).

CA was found to reduce oxidative stress in mouse hippocampus. A dosage of 300 mg/kg of CA significantly decrease the levels of an oxidative stress biomarker, 4-hydroxynonenal, in the mouse hippocampus and the activated microglia indicating that CA has potential to maintain brain health through its antioxidant capacity (Koga et al. 2019).

The biological activity of FA was first found in 1970, and now the antioxidant activity of FA is one of the most well-known activities. Because of the extended side chain and phenolic nucleus present in FA, it easily forms resonance-stabilized phenoxy radicals that mainly responsible for the free radical scavenging activity (Zhao and Moghadsian 2008). This facilitates FA to protect lipids and deoxyribonucleic acid against oxidation through ROS. In addition to ROS, FA is also reported to enhance the expression level of superoxide dismutase, catalase, and antioxidant enzyme. Recently, scientists reported the high efficacy of FA and its derivatives in reducing cyclooxygenase (COX) activity and xanthine oxidase effect.

Table 2 Selected bioactivity studies of CGA, CA, and FA and its derivatives in animals

Compounds	Animal model	Key findings	References
Chlorogenic acid (GCA)	Rat	Treats diabetic nephropathy by inhibiting oxidative stress and inflammation through modulation of the Nrf2/HO-1 and NF-κB pathways	Bao et al. (2018)
	Pig	CGA reduced weaning stress-induced diarrhea It enhanced the growth performances of weaned pigs through maintaining antioxidant capacity and intestinal digestion and absorption function	Chen et al. (2018a)
	Mice	CGA significantly decreased serum triglyceride, cholesterol, hepatic lipids, fasting blood glucose, inflammation, hyperinsulinemia, and hyperglycemia It increased insulin sensitivity It improves high-fat diet-induced hepatic steatosis and insulin resistance in mice	Ma et al. (2015)
	Rat	CGA showed its beneficial role on wound repair and diabetes under diabetic conditions	Bagdas et al. (2015)
	Rat	CGA effectively inhibited isoproterenol-induced hypertrophy in neonatal rat myocytes	Li et al. (2014)
	Mice	Showed protective effect on tetrachlorobenzoquinone-induced liver injury	Xu et al. (2014)
	Rat	It controlled high levels of cholesterol considerably by upregulating the gene expression of PPAR-α in hypercholesterolemic rats induced with a high-cholesterol diet	Wan et al. (2013)
	Mice	CGA reduced heart triglyceride levels and ameliorate cardiovascular disorders It exhibited anti-obesity property and improved lipid metabolism in high-fat diet-induced obese mice	Cho et al. (2010)
Caffeic acid (CA)	Rat	CA exhibited antidiabetic nephropathy effect and mechanism of action Reactivation of autophagy pathway by suppression of autophagy regulatory miRNA	Matboli et al. (2017)
	Rat	CA suppressed hepatic inflammation, oxidative damages, hepatocyte apoptosis, and microcirculatory disturbance caused due to I/R in rat liver	Mu et al. (2015)
	Rat	CA and CGA prevented diabetic-induced brain disorder by inhibiting acetylcholinesterase activity, reducing anxiety, improving memory in diabetic rats	Stefanello et al. (2014)
	Mice	CA and 5-CQA inhibited P-selection expression on platelets and decreased the	Park (2009)

(continued)

Table 2 (continued)

Compounds	Animal model	Key findings	References
		platelet activation 5-CQA and CA inhibited COX-1 and COX-2 enzyme activity	
Ferulic acid (FA)	Rat	FA in a combined form with metformin alleviated the symptoms of diabetes in obese rats FA alleviated lipid peroxidation in diabetic rats by changing the expression of pro-inflammatory cytokines, apoptosis, and altering oxidative stress	Nankar et al. (2017)
	Rat	FA showed antidiabetic effects by modulating insulin signaling molecules in the liver of high-fat diet and fructose-induced type 2 diabetic rats	Narasimhan et al. (2015)
	Isolated rat hepatocytes (Ex vivo)	Polyphenols including CA, FA, ethyl ferulate, methyl ferulate exhibited hepatoprotective effects in different models of rat hepatocytes	Maruf et al. (2015)
	Rat	FA-treated wounds epithelized faster in an excision diabetic wound model	Ghaisas et al. (2014)
	Rabbits	Sodium ferulate inhibited atherosclerogenesis in hyperlipidemia rabbits	Wang et al. (2004)

Hence it is believed that FA lessened a number of oxygen species produced by the enzyme-catalyzed transformation (Nile et al. 2016). Moreover, FA also shows promising effects for the treatment of disorders associated with oxidative stress such as Alzheimer's. These wide ranges of antioxidant capacity seem to impel CGA, CA, and FA to act as an effective nutritional supplement for human and animal health.

21.4.2 Antimicrobial Effect

The researches related to the antimicrobial effect are mostly in vitro study. Various studies showed that CGA inhibited microorganisms that grow throughout human GI track, from the mouth to colon (Farah and Lima 2019). Generally, the antimicrobial activity of CGA, FA, and CA on different microorganisms depends on the position and number of substitutions in the benzene ring and the saturated side chain length. Phenolic acids and their derivatives with longer alkyl chain showed greater antimicrobial activity. CGA and CA present in coffee showed inhibitory effect against pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella choleraesuis* (Martinez-Tome et al. 2011). CGA also effectively inhibited the growth of bacterial strains such as *Shigella dysenteriae* and *S. pneumonia* by provoking

irreversible permeability changes in the cell membrane, causing cell death (Lou et al. 2011; Barbieri et al. 2017). Suarez-Quiroz et al. (2013) found that green coffee CGAs have an important antibacterial, antifungal, and anti-mycotoxigenic effects. FA showed bacteriostatic and bactericidal activities against *Escherichia coli*. The antimicrobial mechanisms of CGA included binding to and disruption of the outer cell membrane followed by the release of cytoplasm macromolecules which leading to cell death (Lou et al. 2011).

21.4.3 Cardioprotection Ability

Cardiovascular diseases (CVD) have claimed the first spot as the leading cause of death in many countries across the world and particularly in developed countries. CVD is a chronic disease that remains prevalence for decades, and the phenolic acids such as CGA, CA, and FA have found as a remarkable role to ameliorate such diseases. Oral administration of CGA and CA to mice inhibited P-selection expression on platelets, which in turn decreased the platelet activation. In addition, CGA and CA were able to inhibit COX-1 and COX-2 enzyme activity (Park 2009). Cho et al. (2010) found that CGA reduced heart triglyceride levels and ameliorated cardiovascular disorders. CGA effectively inhibited isoproterenol-induced hypertrophy in neonatal rat myocytes (Li et al. 2014). CGA has the capacity to control high levels of cholesterol considerably when it was consumed as a dietary supplement (Cho et al. 2010; Wan et al. 2013). FA has been reported for the treatment of the number of chronic diseases including cardiovascular diseases. Sodium ferulate, a salt of FA, has been used in China to treat cardiovascular and cerebrovascular diseases (Wang et al. 2004).

21.4.4 Neuroprotection Effect

Oxidative stress has been known as responsible for the neurotoxicity and pathology associated with various neurodegenerative diseases (Alzheimer's disease), wherein the free reactive nitrogen species (RNS) and ROS generated in the brain can lead to lipid peroxidation or RNA, DNA, and protein oxidation and neuronal dysfunction or even death (Gohil et al. 2012). Coffee constituents such as CGA, CA, and FA are known potent scavengers of ROS and RNS, thereby inhibiting the free radical attack DNA/RNA/proteins, hence preventing their oxidative modification in the brain. CGA and CA demonstrated the ability to prevent diabetic-induced brain disorder evidenced by inhibiting acetylcholinesterase activity, reducing anxiety, and improving memory in diabetic rats (Stefanello et al. 2014).

FA could be potentially used as a therapeutic molecule for nucleus pulposus (NP) regeneration. Protective effects of FA ethyl ester against the amyloid beta peptide (1-42)-induced oxidative stress and neurotoxicity have been evaluated (Sultana et al. 2005). They reported the potential importance of FA ethyl ester to treat Alzheimer's disease and other oxidative stress-related diseases. Another study showed the

neuroprotective effects of FA toward oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting ICAM-1 mRNA expression in rats. These results infer the promising neuroprotection effect of CGA, CA, and FA in various in vivo and in vitro systems (Cheng et al. 2008).

21.4.5 Hepatoprotection Capacity

CGA has protective effects on tetrachlorobenzoquinone (TCBQ)-induced liver injury. Xu et al. (2014) found that pretreatment of CGA relieved TCBQ-induced oxidative stress and liver damage significantly indicating the hepatoprotective nature of CGA. Sun et al. (2014) reported a protecting ability of CGA in a dose-dependent manner toward carbon tetrachloride (CCl_4)-induced acute liver injury in male Sprague Dawley rats.

Mu et al. (2015) has described the therapeutic role of CA against I/R-induced hepatic injury in rats. They found that CA suppressed hepatic inflammation, oxidative damages, hepatocyte apoptosis, and microcirculatory disturbance in rat liver. Phenolic constituents such as CA, FA, and their derivatives showed efficient hepatoprotective effects against methylglyoxal/glyoxal provoked oxidative damage and cytotoxicity (Neelam and Sharma 2019). The hepatoprotective effect follows the order: CA > FA > ethyl ferulate > methyl ferulate in different models of rat hepatocytes (Maruf et al. 2015). FA has also been found to have a hepatoprotective effect against CCl_4-induced acute liver injury through suppression of inflammatory signaling pathways and oxidative stress (Kim et al. 2011). These results implied that CGA, CA, and FA can serve as potential hepatoprotective candidates for liver injury.

21.4.6 Antidiabetic and Antilipidemic Activity

Noncommunicable diseases are the most common causes of morbidity or premature mortality, and diabetes is one of them. It is the 4th or 5th leading cause of death in many countries. CGA is a potential antidiabetic candidate. It has been reported that coffee consumption lowered the possibility of type 2 diabetes (Van Dam 2008). Ong et al. (2013) found that the chronic administration of CGA inhibited hepatic glucose-6-phosphatase expression and activity, improved lipid profiles, attenuated hepatic steatosis, and enhanced skeletal muscle glucose consumption, which in turn enhanced insulin sensitivity, dyslipidemia, glucose tolerance, and fasting glucose level in $Lepr^{db/db}$ mice. Green coffee bean extract reduced visceral fat and body weight in mice. High-fat diet rats fed with CGA (100 mg/kg) significantly decreased serum triglyceride, cholesterol, hepatic lipids, fasting blood glucose, inflammation, hyperinsulinemia, and hyperglycemia and increased insulin sensitivity (Ma et al. 2015). Another study found that high-fat diet rats fed with green coffee bean extract had a significantly lower fat mass and body weight compared with control group (Choi et al. 2016).

Recently, Matboli et al. (2017) examined the antidiabetic nephropathy effect of CA and proved its mechanism of action that occurred by reactivation of autophagy pathway via suppression of autophagy regulatory miRNAs. FA showed antidiabetic effects by modulating insulin signaling molecules in the liver of high-fat diet and fructose-induced type 2 diabetic rats. FA-treated rats illustrated the downregulation of glucose 6-phosphatase, phosphoenolpyruvate carboxykinase, and gluconeogenic enzyme genes, thereby reducing gluconeogenesis (Narasimhan et al. 2015). FA promoted wound healing effect in streptozotocin-induced diabetic rats. FA also was found to alleviate the symptoms of diabetes in obese rats and reduce lipid peroxidation in diabetic rats through changing the expression of pro-inflammatory cytokines, apoptosis, and altering oxidative stress (Nankar et al. 2017).

21.4.7 Anti-inflammatory Activity

Coffee promotes a lower risk of chronic conditions such as reducing inflammation, oxidative stress, and increasing serum levels of anti-inflammatory factors like adiponectin and interleukins (Cadona et al. 2019). The role of CGA in controlling oxidative and inflammatory conditions has been reported (Liang and Kitts 2016). Chauhan et al. (2012) found that oral administration of 40 mg/kg CGA to lipopolysaccharide (LPS)-induced arthritis rat inhibited pro-inflammatory cytokines, thereby exhibiting the enhanced healing of tissue damage caused by inflammation. CGA protected against trinitrobenzene sulfonic acid-induced colitis in mice through decreasing neutrophil infiltration and inhibiting nuclear factor kappa B (NF-κB) pathway (Zatorski et al. 2015).

Caffeinated and decaffeinated coffees act as anti-inflammatory agents in therapeutic processes in various pathological situations. Coffee's therapeutic potential results from its bioactive substances including caffeine, CGA, and CA. Together, these compounds have been exhibited to modulate inflammatory, to accelerate wound healing in neuropathic pain and to reduce inflammation in animal models (Leon et al. 2019). An anti-inflammatory activity of CA in cardiac tissue of diabetic mice has been evaluated by Chao et al. (2009). They reported that CA could provide anti-inflammatory, antioxidative, triglyceride-lowering, and anticoagulatory protection in cardiac tissue of diabetic mice. Hence, the supplement of CA is helpful for the prevention/attenuation of diabetic cardiomyopathy. In an excision wound model, FA-treated wounds were found to epithelize faster compared with diabetic wound control group (Ghaisas et al. 2014).

21.5 Benefits (Human Studies)

21.5.1 Antioxidant Activity

Coffee constituents like CGA, CA, and FA have been revealed to be an effective antioxidant in human studies. The possible reason for the potent activity of these phenolic acids is due to its capability to induce upregulation of cytoprotective

enzymes (extracellular signal-regulated kinase ½, heat shock protein 70, Akt, and heme oxygenase-1), to scavenge free radicals, and to lessen the activity and expression of cytotoxic enzymes (COX-2, inducible nitric oxide synthase (iNOS), and caspases) (Chen et al. 2014). A clinical study conducted by Hoelzl et al. (2010) showed that individuals injected instant coffee that included green and roasted coffee bean extracts which contained 300 mg CGA significantly decreased two of the oxidative stress biomarkers in urine, 8-isoprostaglandin F2α and 3-nitrotyrosine, by 15.3% and 16.1%, respectively, when compared with the control group who only consumed water.

21.5.2 Antidiabetic Activity

Prospective cohort investigations of coffee intake and risk of type 2 diabetes mellitus have been studied widely in various countries including the United States, Sweden, Finland, and the Netherlands. These studies have established a significant inverse association between coffee consumption and risk of type 2 diabetes mellitus (Higdon and Frei 2006). A cohort including more than 193,000 men and women showed that those who consumed 6 cups or 2 cups of coffee daily reduced the risk of type 2 diabetes mellitus 35% and 28%, respectively (Van Dam and Hu 2005). The antidiabetic activity of coffee is strongly related to the presence of phenolic compounds especially CGAs in coffee, and the mechanisms included the reducing glucose production from the liver by phenolic acids; inhibiting glucose-6-phosphate translocase thus reducing glucose absorption; inhibiting gut incretin hormones; and protesting pancreatic β-cells against oxidative stress (Gokcen and Sanlier 2019; Van Dam and Hu 2005).

It was found that green coffee extract which contains mainly CGAs reduced glucose-6-phosphatase hydrolysis and inhibits the generation of hepatic glucose, thereby showing antidiabetic effect (Henry-Vitrac et al. 2010). Cheng et al. (2011) reported that consumption of CA can hinder the misfolding of human amylin polypeptide, thus potential for ameliorating type 2 diabetes. A few clinical pharmacokinetics studies of FA in humans have been reported in the literature to show its antidiabetic activity (Mancuso and Santangelo 2014).

21.5.3 Anti-obesity Activity

CGA, CA, and FA show anti-obesity activity by decreasing cholesterol level as well as fatty acid biosynthesis (Gokcen and Sanlier 2019). Further, it altered the plasma adipokine levels while enhanced fatty acid oxidation and PPAR alpha expression in the liver (Cho et al. 2010). A study demonstrated that compared with normal instant coffee, CGA-enriched instant coffee had a significant role in the utilization and absorption of glucose from the diet and reduced body fat and body mass, remarkably (Thom 2007). Another study elaborated on the differences in insulin, gastrointestinal hormone profiles, and plasma glucose and further confirmed the antagonistic effect of CGA on glucose transport (Johnston et al. 2003). Overweight and

obese individuals who consumed coffee containing green coffee bean extract have a decline body weight, and 80% of the weight loss was due to the fat loss (Thom 2007).

21.5.4 Antiviral Activity

CGA, quinic acid, and CA have been reported to possess anti-hepatitis B virus activity, and CGA derivative, caffeoyl 5,6-anhydroquinic acid, exhibited significant anti-HIV capacity (Chaves-Ulate and Esquivel-Rodríguez 2019).

21.5.5 Anticancer Activity

Although many studies have found the positive association between coffee consumption and the risk of various cancers, epidemiological studies investigating the role of coffee consumption on cancer still could not obtain a conclusive answer. Human studies have revealed that coffee consumption has the efficiency of preventing breast cancer, prostate cancer, colon cancer, hepatocellular carcinoma, and rectal cancer. In vivo and in vitro investigations have established the anti-carcinogenic activity of CA against hepatocellular carcinoma (HCC) which is the most common type of primary liver cancer, considered to be of vastly aggressive, high incidence and causing substantial mortality across the world. In addition, these studies elaborated that the intake of CA-abundant foods showed a protective activity toward carcinogenesis by inhibiting the generation of nitrosamides and nitrosamines. These nitro compounds are the major inducers of pathology. The anticancer ability of CA is connected with its antioxidant effect which is ascribed to its molecular structure that has double bond in the carbonic chain, number of hydroxyl group, different positioned hydroxyls in catechol group, and free phenolic hydroxyls (Espindola et al. 2019).

21.5.6 Antihypertension

The impacts of CGA-enriched green coffee on blood pressure have been summarized by Sanlier et al. (2019). Kozuma et al. (2005) found that when mild hypertension Japanese subjects consumed 93 mg or 185 mg green coffee bean extract, which equivalent to 50 mg or 100 mg CGA for 28 days, their systolic and diastolic blood pressures were reduced. The possible antihypertension reasons are that bioactive component such as CGA in green coffee inhibited 11β-HSD1 enzyme activity, thus decreasing the ratio of cortisol/cortisone (Revuelta-Iniesta and Al-Dujaili 2014); CGA increase nitric oxide bioavailability; the metabolite of CGA, ferulic acid, reduced oxidative stress by attenuating superoxide (Watanabe et al. 2006).

21.6 Application in Food (Including Correctly Cooking Foods Rich in Phytochemicals)

Owing to the enormous biological applications of CGA, CA, and FA, they gained considerable attention in food industries. As a result, several dietary foods that have these major phenolic acids both in combined with other compounds and individual forms are commercially available in the market. Due to its antimicrobial and antioxidant potential, it has been used as a food preservative as well. Green coffee extracts which is rich in CGAs, CA, and FA have been used to fortify different foods or make dietary supplements. Ground coffee bean powder was added into the bread to make phenolic enrich products that retained greater antioxidant activities (Dariusz et al. 2015). Green coffee extract enriched soy milk was found to have increased antioxidant activity, high bioaccessibility, and increased protein and starch digestibility (Seczyk et al. 2017). CGAs, the main precursors of coffee flavor and colors, contribute to the final acidity and bitterness of coffee. During roasting of coffee beans, CGAs undergo dehydration, fragmentation, Strecker degradation, and Maillard reactions to produce CA, low-molecular-weight compounds, and quinolactones which cause the increase of bitterness of coffee. The beans of *C. arabica* contain lower CGAs compared with *C. canephora*, which causes the better cup quality of *C. arabica* bean. However, CGAs in coffee leaves are more abundant in *C. arabica* than in *C. canephora*, thus tending to produce greater bioactivity tealike beverage when using the former source. Therefore, the selection of coffee species and the administration of roasting conditions are crucial in order to produce high quality of coffee. When production of green coffee extract as supplement is considered, it is a good choice to use the *C. canephora* beans that contain more CGAs.

CA was used to modify ovalbumin, which can reduce the allergenicity of ovalbumin (Tong et al. 2018). CA-incorporated film showed a 6-fold increased antibacterial activity and 20-fold enhanced antioxidant activity compared with CA-free composite films (Yu et al. 2013). The releasable CA exhibited considerable inhibitory ability on lipid oxidation of menhaden oil-in-water emulsion. CA and FA effectively inhibited the lipid oxidation which plays a key role to produce rancidity and off-flavor in frozen minced fish (Medina et al. 2009). Takahashi et al. (2013) reported that FA had the ability to inhibit the growth of *L. monocytogenes* (food-borne pathogenic bacteria) in cheese and smoked salmon. A soy protein isolate-based edible coating containing FA controlled browning index, weight loss, and firmness of fresh-cut apples when stored at the temperature of 10 °C and the relative humidity of 50%; therefore FA prolonged the shelf life and enhances the quality of freshly cut apple (Alves et al. 2017). In Japan, FA has been permitted as a food preservative and additive antioxidant due to its antioxidant and antimicrobial properties. FA has been added to sports foods to enhance athletic performance. For example, as a sport supplement, FA can reduce fatigue and muscle tenderness due to its free radical scavenging activity (Tee-Ngam et al. 2013). Due to the unique properties and applications, FA has received considerable interest in oriental

research where it widely used as an efficient antioxidant. Dietary supplementation with FA enhanced the meat quality and the antioxidant activity of poultry by decreasing serious level of MDA and increasing the hepatic activity of GSH-Px (Saeed et al. 2019).

21.7 Safety: Toxicity and Side Effects

The researches related to the toxicity and side effects of CGA, CA, and FA are very scant, especially for animal and clinical studies. A study done by Moridani et al. (2001) using isolated rat hepatocytes showed that CA and CGA can metabolize into cytotoxic o-quinone by cytochrome P450 and CA has higher cytotoxicity than CGA. Although the health benefits of CGA-rich coffee or green coffee bean have been widely reported, there are studies also related these compounds to the risk of chronic diseases to coffee consumption (Sanlier et al. 2019). Excessive consumption of coffee leads to the possibility of cardiovascular disease that is probably due to the occurrence of cholesterol-raising agents (Butt and Sultan 2011). Healthy individuals supplement with 2 g CGA per day for 7 days were found to have higher (>12%) total homocysteine in the postprandial plasma compared with placebo group, indicating the possible risk of CGA to the cardiovascular diseases (Olthof et al. 2001b). Although researchers have found that green coffee could reduce blood pressure in the mild hypertension Japanese patients (Kozuma et al. 2005), consumption of green coffee extract which contains 140 mg CGA for 12 weeks significantly decreased systolic and diastolic blood pressure in health individuals, suggesting the possible hypotension risk (Watanabe et al. 2006). Liu et al. (2019) found that mice consumed 5 or 150 mg/kg/day of caffeic acid before gestation affected implantation of embryos and the higher dose caused the low fetal weight. Although CGA is generally considered as an antioxidant, it can turn into prooxidant at high concentration and in the presence of transition metal ion.

21.8 Marketed Products

A wide range of dietary supplements containing green coffee extracts is commercially available in the market. They are offered in various forms which include powder, tablet, and capsule. Green coffee extract supplements generally contain about 50% CGAs and have claimed to help weight loss, suppress appetite boosts, control blood sugar level, and maintain heart health. For example, Svetol® (Naturex) is a decaffeinated green coffee extract supplement that has a specific ratio of CGA and its derivatives with a claimed slimming function (Dellalibera et al. 2006; Henry-Vitrac et al. 2010). Body fat and weight-reduced coffee extract supplements are also available (Thom 2007). Other products contain green coffee bean extract which exhibits antihypertensive potential are available in the combined form of Svetol® with olive leaf extract and beet powder (Wong et al. 2014). Lisopresol® is a chewing gum containing green coffee extract, L-carnitine, and nutraceutical

products that might aid in the control over snack intake and reduce hunger sensations (Bobillo et al. 2016). Green coffee bean extract has also been made into GRECOBE-Green Coffee Bean Extract sachets which can be consumed with hot water infusion. Ground green coffee bean powder can also be packed in sachets to make green coffee infusion. The concentration of CGAs ranged from 628 to 1040 mg/L in *C. arabica* hot water infusion and from 682 to 1210 mg/L in *C. canephora* infusion when 3 g green coffee bean powder infused with 200 ml boil water (Macheiner et al. 2019). Coffee leaves are also made into different types of coffee leaf teas (Chen et al. 2018b) and Kombucha with different flavors. FA has the ability to neutralize free radicals in muscle tissue; thereby it treats muscle fatigue, and hence it is widely used as a sports supplement.

21.9 Patents

CGA, CA, FA, and their derivatives are subjects of numerous patent submissions around the world. The search engine for patents by Google patent showed more than 55,000 patents related to them. The preparation of phenolic compounds particularly CGA, CA, and FA from green coffee extract with various forms has also been widely patented (US20160030350A1, US20180235251A1, TW201440781A, KR101999108B1, CN1813556A, US10335444B2, CN105175266A). The patents list a wide range of extraction protocols of these polyphenolic compounds from green coffee bean (CN106278891A, CN101404891B, KR102047346B1, JP5295559B2), roasted coffee bean (JP2017093301A, JP6139724B2), coffee (JP2018529378A, US10449225B1), coffee fruit extract (KR20180054660A), coffee decaffeination with caffeic acid (CA1320658C, US4767634A, EP0340354A1), guava leaf (CN108498582A), tobacco leaves (CN109696514A), apple (CN109123321A), olive (CN109030664A), kiwifruit (CN108680660A), and other plant residues (ES2569132B1).

A number of patents are available to describe the various applications of phenolic acid that consist of CGA, CA, and FA in coffee especially antioxidant activity (CN105494825B). An effect of a decaffeinated green coffee extract on body weight control has been developed by regulation of glucose metabolism (US9358264B2). CGAs were often used as active agents for slimming in cosmetics (FR2883472A1), flavor modifier (ES2247339T3, TWI283168B), and taste modification (WO2019040989A1, JP5766173B2) in beverages. Water-soluble green coffee bean extract consists of 70% CGAs, and $<1\%$ caffeine has been developed as an antioxidant supplement and is effective in healthy weight management (PCT/IN2015/000236). Antioxidant containing these phenolic acids as an effective ingredient was described widely (KR20050110186A, JP3855293B2, WO2006103515A1, CN105494825B). Antihypertensive agent (JP4119629B2) that containing those bioactive constituents showed a significant effect in hypertension (US7939563B2, US7351436B2) or preventing a rise in blood pressure (EP1186294A2).

In pharmaceutical field, these bioactive compounds show various health benefits, including the treatment of aging, age-related disorders, and/or age-related manifestations including atherosclerosis, peripheral vascular disease, coronary artery disease, osteoporosis, arthritis, type 2 diabetes, dementia, Alzheimer's disease, and cancer (US20060275294A1, US20190374489A1, WO2014048888A1, JP4713765B2, CN105076916A, CN103068359A, CA2685031A1, US20060172012A1, JP6406730B1, US20160296490A1, US20160193306A1).

21.10 Perspectives

CGAs, CA, and FA received considerable attention due to their wide distribution in plant foods and potential health benefits. Although a number of in vitro studies have shown various biological activities of these compounds, the in vivo human studies are still scant. Most of the clinical studies focus on the health benefits of coffee or green coffee beans and lack of the researches on the in vivo bioactivity of pure compounds. Among this group of phenolic acids, majority of the researches are related to the CGA (5-CQA), and researches related to other minor CGA derivatives are very scant. Since food is a very complex system, food matrix has a great impact on the bioaccessibility, bioavailability, and bioefficacy of bioactive compounds. Coffee contains different groups of bioactives; therefore, it is hard to make a conclusive proof for the health benefits of CGA alone in the clinical studies when individuals are administrated coffee. Therefore, understanding the physical and chemical characteristics of macromolecules such as polysaccharide- and protein-phenolic acid conjugates in different food systems, the interaction of other groups' phytochemicals with phenolic acids, and the impacts of macromolecules and other bioactives to phenolic acids are crucial to better utilize CGAs, CA, and FA and to guide the processing methods. Moreover, the bioefficacy of bioactives is largely varied in individuals. Therefore, precision impacts of bioactives need to be considered in future researches using more advanced analytical techniques and data processing methods.

Processing, especial thermal processing, not only affects the organoleptic characteristics of food but also influences the content and structure of phenolic compounds. For example, about 60–98% of CGA is isomerized or transformed into quinolactones in different roasting processes. Thus, a large quantity of thermal processing products such as 3-caffeoylquinic-1, 5-lactone (3-CQL), and 4-caffeoylquinic-1, 5-lactone (4-CQL) produced in roasted coffee bean. Therefore, more attentions need to be paid to the bioavailability and bioefficacy of the major processing products in order to better understanding the benefit and risk effects of injected foods. Moreover, the injected CGAs, CA, and FA undergo extensive transformation to produce various metabolites; thus it is difficult to distinguish the efficacy of parent phenolic compounds from the metabolic compounds. More researches are needed to be done using the metabolites to elucidate the protective mechanisms of food origin bioactives in human body.

21.11 Cross-Reference

► Coumaric and Cinnamic Acids in Food

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Caffeoylquinic Acids

22

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Contents

Abstract

Caffeoylquinic acids (CQAs) are a large family of phenylpropanoids (chemically esters) found in large amounts in coffee beans but typically occur in many other edible plants, and as such, they belong to the most widespread polyphenols in plant kingdom. They are also associated with significant health benefits resulting from the consumption of beverages such as coffee and yerba maté as well as some popular vegetables such as globe artichoke or endive. Also, many medicinal herbs

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owe their properties to their CQA content. Here, we focused on the importance of these compounds for functional properties of healthy foodstuff, summarizing such well-studied aspects as bioavailability, in vitro and in vivo pharmacological effects, and potential for lifestyle disease prevention and management. The chemical diversity and analytical approaches are also outlined.

From the literature review, it is concluded that CQAs count as the most essential healthy constituents of various foods and beverages and should be included in characterization of edible consumer products. At the same time, one should remember that caution is necessary in claiming unfounded properties to cure diseases. Therefore, more research is necessary, especially on human subjects, as well as further development of affordable and reliable analytical methods in an ultimate goal to understand their distribution, diversity, and function.

Keywords

Chlorogenic acids · Antioxidant · Coffee · Artichoke · Asteraceae · Hydroxycinnamate

22.1 Introduction: What Are Caffeoylquinic Acids?

Phenolic acids are widely distributed in various foodstuffs of vegetables, but a significant proportion of them occurs as esters or depsides. Among them, one of the best known groups is conjugates with (−)-quinic acid, known collectively as chlorogenic acids (coming from chlorogenic acid, CGA, the most widespread and the first to be described compound of their kind). A number of related structures formed with other hydroxylated organic acids are sometimes being included in this category. The diversity and distribution of various hydroxycinnamic-acyl conjugates have been extensively studied, discussed, and reviewed by Clifford and co-workers in several papers that have been appearing since more than two decades (Clifford 1999, 2000, 2017; Clifford et al. 2007, 2017; Abrankó and Clifford 2017).

They occur in fruits, vegetables, and beverages such as coffee, yerba maté, and wine. Chlorogenic acids are extremely widely distributed among higher plants and in some cases are found in surprisingly high concentrations (Azuma et al. 2000; Clifford 2017).

Many of these compounds, like other polyphenols, are also associated with important health benefits, especially in the context of lifestyle disease prevention. Consequently, a lot is being spoken about how coffee is good for you and why people should consume a lot of vegetables from the Compositae (Asteraceae) botanical family – artichokes, endive, radicchio, etc.

Chlorogenic acid, contained in almost every studied higher plant species, is the most typical example and one of the most intensively studied plant dietary

polyphenols. In fact, most of the pharmacological and physiological activities of CQAs were described for CGA.

22.2 Chemistry of CQAs

They are formed by condensation between (−)-quinic acid [1 L-1(OH),3,4/5-tetra-hydroxycyclohexane carboxylic acid according to IUPAC and certain *trans*- acids, usually caffeic (CQAs) (see Table 1). Quinic acid has axial hydroxyl groups on carbons 1 and 3 and equatorial hydroxyl groups on carbons 4 and 5. During processing or upon UV-irradiation, *trans* isomers of cinnamic derivatives may be partially converted to *cis* form (Clifford 1999). CQAs are categorized according to the number or the position of caffeoyl groups and their additional modifications (Miyamae et al. 2011). Among all CQAs, 5-*O*-caffeoylquinic acid (5-CQA) is the most ubiquitous compound in the plant kingdom, known as chlorogenic acid. 3-*O*-

Table 1 Positional variants of caffeoylquinic and caffeoylshikimic acids

Name and abbreviation		R1	R3	R4	R5
Quinic acid	QA	H	H	H	H
1-*O*-Caffeoylquinic acid	1-CQA	C	H	H	H
3-*O*-Caffeoylquinic acid	3-CQA	H	C	H	H
5-*O*-Caffeoylquinic acid	5-CQA	H	H	H	C
4-*O*-Caffeoylquinic acid	4-CQA	H	H	C	H
1,3-Di-*O*-caffeoylquinic acid	1,3-diCQA	C	C	H	H
1,4-Di-*O*-caffeoylquinic acid	1,4-diCQA	C	H	C	H
1,5-Di-*O*-caffeoylquinic acid	1,5-diCQA	C	H	H	C
3,4-Di-*O*-caffeoylquinic acid	3,4-diCQA	H	C	C	H
3,5-Di-*O*-caffeoylquinic acid	3,5-diCQA	H	C	H	C
4,5-Di-*O*-caffeoylquinic acid	4,5-diCQA	H	H	C	C
1,3,4-Tri-*O*-caffeoylquinic acid	1,3,4-triCQA	C	C	C	H
1,3,5-Tri-*O*-caffeoylquinic acid	1,3,5-triCQA	C	C	H	C
1,4,5-Tri-*O*-caffeoylquinic acid	1,4,5-triCQA	C	H	C	C
1,3,4,5-Tri-*O*-caffeoylquinic acid	1,3,4,5-tetraCQA	C	C	C	C
		R1	**R3**	**R4**	**R5**
Shikimic acid	SA				
3-*O*-Caffeoylshikimic acid	3-CSA	–	C	H	H
5-*O*-Caffeoylshikimic acid	5-CSA	–	H	H	C
4-*O*-Caffeoylshikimic acid	4-CSA	–	H	C	H
3,4-Di-*O*-caffeoylshikimic acid	3,4-diCSA	–	C	C	H
3,5-Di-*O*-caffeoylshikimic acid	3,5-diCSA	–	C	H	C
4,5-Di-*O*-caffeoylshikimic acid	4,5-diCSA	–	H	C	C

caffeoylquinic acid (3-CQA), also called neochlorogenic acid, and 4-*O*-caffeoylquinic acid (4-CQA), known as cryptochlorogenic acid, frequently accompany the former compound.

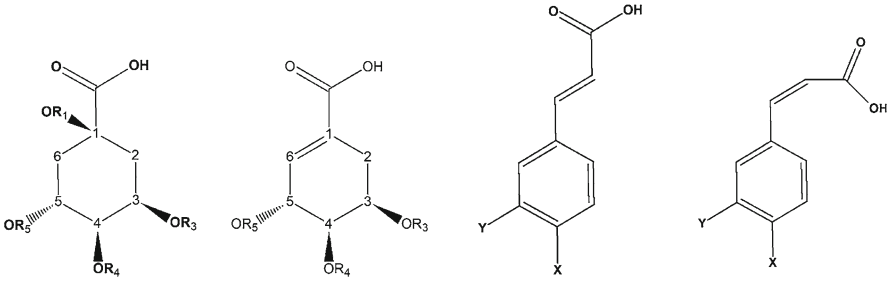
Moreover, (−)-quinic acid can be esterified with more molecules of caffeic (or other hydroxycinnamic) acid, and the following subgroups are known: dicaffeoylquinic acids (diCQAs), for example, in *Lonicera japonica* (Yang et al. 2018) or *Artemisia campestris* (Pereira et al. 2018); tricaffeoylquinic acids (triCQAs) in *Ipomoea aquatica* (Lawal et al. 2017) or in *Solidago* sp. (Abdel Motaal et al. 2016; Woźniak et al. 2018); and even tetraCQAs in *Bridelia ferruginea* stem bark (Cimanga et al. 1999). We also know mixed diesters of caffeic and ferulic acids, i.e., caffeoyl-feruloylquinic acids (CFQAs), which are characteristic of robusta coffee (*Coffea canephora*, Rubiaceae) or caffeic and sinapic acids, i.e., caffeoyl-sinapoylquinic acids (CSiQAs), as found in *Gardenia jasminoides* (Rubiaceae) (Nishizawa et al. 1987), *Broccoli* sprouts (Moreira-Rodríguez et al. 2017), or *Ilex paraguariensis* (Jaiswal et al. 2010). Mixed esters involving various combinations of one to three residues of caffeic acid with one or two residues of a dibasic aliphatic acid like glutaric, oxalic, or succinic are also known, especially in the Asteraceae family (Clifford et al. 2008; Maruta et al. 1995). Galloyl conjugates of quinic acids and cinnamoyl conjugates of quinic acid derivatives such as shikimic acid methyl or butyl quinate, and 4-deoxyquinic acid also occur in certain plant species. For example, caffeoylshikimic derivatives 3-QSA and 4-QSA are found in nectar of many flowering plants, among them *Vaccinium corymbosum* L., where they serve as a natural pathogen defense and influence plant-pollinator interaction (Egan et al. 2018).

Another kind of hydroxycinnamate conjugates forms when quinic acid is replaced by:

1. Other hydroxylated organic acids, mainly α-hydroxyhydrocaffeic, malic, and tartaric but also galactaric, glucaric, gluconic, hydroxycitric, methoxyaldaric, phenylpyruvic, and tartronic.
2. Amides of amino compounds including aromatic amino acids, choline, anthranilic acids, and diamines.
3. Esters of polysaccharides, simple sugars, and sugar alcohols including glycerol and *myo*-inositol. Especially chlorogenic acid glycosides were described with glucosyl or rhamnosyl carbohydrate moiety, glycosides including those of anthocyanins, flavonols, and diterpenes.
4. Esters of lipids including alkanols, alkandiols, u-hydroxy-fatty acids, and sterols (Zhang et al. 2013; Clifford 2000; Baeza et al. 2016; Alonso-Salces et al. 2009; Vo et al. 2014).

Different methoxy derivatives such as mono-, di-, or tri-methoxycinnamic and sinapic acid residues are described too (Kargutkar and Brijesh 2018; Sytar et al. 2018; Zengin et al. 2018). Chlorogenic acids are relevant coffee quality markers, and more than 70 chlorogenic acid derivatives have been detected so far in green coffee (Alonso-Salces et al. 2009; De Rosso et al. 2018). As we mentioned before, these

classic CGAs may be subdivided by the identity, number, and position of acyl residues as follows (Nagy and Abrankó 2016):



QA = quinic acid **SA**-shikimic acid **C** = cinnamic acid (trans) (cis)

X = H, **Y** = OH p-coumaric acid;

X = **Y** = OH caffeic acid;

X = OCH3, **Y** = OH ferulic acid

It is widely accepted that the known biosynthetic pathway produces *trans* form of cinnamic derivatives, and these dominate in most extracts; also it has been long known that conversion to the *cis* form occurs readily, especially after UV light exposure (Clifford et al. 2008).

22.2.1 Identification of CQAs

More and more stringent requirements for determining the quality of food products, including those of vegetable origin, have made analytical techniques used for this purpose important and increasingly used as a standard quality control method. Nowadays, there is a general trend in food science that link food and health, considering food as not only alimentation but also an affordable way to prevent future diseases and provide health benefits. Depending on the type and availability of CQAs from the different matrices, various extraction solvents are suggested in the literature; specifically, lower molecular weight polyphenols, such as hydroxycinnamate derivatives, are preferably extracted using methanol (Mané et al. 2007). Methanol use eliminates such extracts from consumption, and for this purpose ethanol or water is more suitable. Non-acidified methanol, ethanol, or water together with suitable extraction time and solid-to-liquid ratio is often chosen to allow a complete extraction of the CQA pool, reducing the risk of their degradation.

Among all analytical techniques, the LC-MS (liquid chromatography-mass spectrometry) is considered well-suited for CQA analysis and identification. To obtain better mass spectra and high-resolution mass spectra, negative ionization mode is recommended for CQA identification (Clifford 2000). Similar spectral behavior and UV detection with maximum absorbance around 300–320 nm and a shoulder around 290–310 nm, characterizing hydroxycinnamic acids and derivatives, facilitate the quick identification of most chlorogenic acids as a class and allow for more

affordable instrumentation to be applied in routine analysis (Crupi et al. 2018). In mass spectrometry-based detection, the typical fragmentor voltage (FV) is varied between 60 and 320 V; values in the range between 60 and 150 V are recommended for small molecules, whereas values above 350 V are only used to fragment large molecules. Cleavage of the hydroxycinnamic acid residue requires less energy if the esterification of QA occurs closer to the carboxyl group, and hydroxycinnamic acid is cleaved easier from position 5 than 3. As a result, regioisomeric monocaffeoylquinic acids (CQAs) and dicaffeoylquinic acid (diCQAs) showed different tandem mass spectra in negative ion mode (Table 3), e.g., producing fragment ions at m/z 191 and 173 corresponded to S1 [quinic acid – H]$^-$ and S2 [quinic acid – H_2O – H]$^-$ ions, respectively, and ions at m/z 179 A1 [caffeic acid –H] characteristic for CQA derivatives with molecular ion (*m/z* 353 λ_{max} 325 nm). Appropriate molecular ions are assigned as follows: for coumaroylquinic acid (*m/z* 337 λ_{max} 315 nm), producing fragment ions at *m/z* 172 and *m/z* 191, 163 corresponded to [quinic acid – H]$^-$ and [coumaric acid – H]$^-$ ions, respectively, feruloylquinic acids (*m/z* 367 λ_{max} 325 nm), sinapic (*m/z* 379 λ_{max} 325 nm), and dicaffeoylquinic acids (*m/z* 515 λ_{max} 325 nm). See (Table 2; Nagy and Abrankó 2016). Conformation *cis* preferably the hydrophobicity compared with *trans* form due to hydrogen bond inside the structure (Clifford et al. 2008).

22.3 Natural Sources of CQAs

Although coffee beans are the most widespread dietary source of these compounds, they are also present, for example, in several vegetables belonging to the Asteraceae (Compositae) family. Globe artichokes and cardoons (*Cynara cardunculus* subsp. *scolymus, C. cardunculus* subsp.*flavescens*) contain cynarin along several other CQA but Jerusalem artichokes (*Helianthus tuberosus*) contain mainly 3,4diCQA isomers.

However, these compounds are ubiquitous among land plants (put together by Clifford 2017), starting from bryophytes, which would be the phylogenetically first plants to contain them, through ferns and horsetails, gymnosperms, and of course flowering plants – angiosperms as the most diverse and advanced group. From cryptogamous taxa (a few mosses; a horsetail, *Equisetum arvense*; several fern species from different families), the most often reported isomer was 5-CQA (neochlorogenic acid). Surprisingly, there are rather few reports on CQAs' presence in gymnosperms, including *Ginkgo biloba* and juniper, and also in berries which are edible and used as spice (Fierascu et al. 2018). Selected examples of plant foods (spices, vegetables, fruits and nuts, beverages) containing CQAs are listed in Table 4, based on Clifford (2017) and some more recent publications (Dillenburg Meinhardt et al. 2019, Jaiswal et al. 2010, Kahle et al. 2005, Maruta et al. 1995, Sytar et al. 2018). Among flowering plants, the monocots seem to be less abundant in CQAs, and their occurrence is rather sporadic.

Table 2 MS^3 data of selected common monoacyl and diacyl hydroxycinnamoyl-quinic acids in negative ion mode

		MS^1			MS^2			MS^3					
		Base peak			Secondary peak			Base peak					
Compound	Parent ion	*m/z*	*m/z*	Int	*m/z*	Int	*m/z*	Int	*m/z*	*m/z*	Int	*m/z*	Int
3-CQA	353.1	191	178.5	50	134.9	7	–	–	85.3	127.0	71	172.9	67
4-CQA	353.2	173	178.9	60	190.8	20	135.0	9	93.2	111.0	48	–	–
5-CQA	353.2	191	178.5	5	135.0	15	–	–	85.2	126.9	66	172.9	27
3-FQA	367.2	193	191.5	2	173.2	2	–	–	133.9	148.9	23	–	–
4-FQA	367.2	173	192.9	16	–	–	–	–	93.1	111.5	44	–	–
5-FQA	367.2	191	172.9	2	–	–	–	–	85.2	126.9	70	–	–
3-pCoQA	337.1	163	190.0	5	–	–	–	–	118.9	–	–	–	–
4-pCoQA	337.1	173	–	–	–	–	–	–	93.0	111.0	61	–	–
5-pCoQA	337.2	191	162.9	5	–	–	–	–	85.2	–	–	–	–
1,3-diCQA	515.1	353	335.1	2	173.0	4	178.9	30	190.9	179.0	60	ND	–
1,5-diCQA	515.2	353	335.1	2	173.0	4	ND	–	190.9	179.0	7	ND	–
3,5-diCQA	515.2	353	335.1	2	173.0	4	ND	–	190.9	179.0	60	ND	–
3,4-diCQA	515.2	353	335.1	4	172.9	20	178.9	15	172.9	178.9	68	191.0	32
4,5-diCQA	515.2	353	335.1	2	172.9	6	178.9	5	172.9	178.9	76	190.9	9

Abbreviations: *F* feruloyl, *pCo* para-coumaroyl, *ND* not detected, *int* intensity

Table 3 Theoretically occurring combinations of quinic acid and the six types of hydroxycinnamic acids including mono-, di-, tri-, and mix-esters in negative ion mode. Dehydroquinic acid (QA-H2O), dehydrocaffeoylquinic acid (CQA-H2O), and dehydroferuloylquinic acid (FQA-H2O) are examples of typical diagnostic ions indicating conjugation at the 4-OH position of quinic acid (Nagy and Abrankó 2016)

No.	Type	Name and abbreviation			Formula	$[M-H]^-$
1		Quinic	Acid	QA	C7H11O6	191.0561
2		Dehydroquinic	Acid	QA-H_2O	C7H9O5	173.0455
3		p-Coumaric	Acid	pCoA	C9H7O3	163.0401
4		Caffeic	Acid	CA	C9H7O4	179.0350
5		Ferulic	Acid	FA	C10H9O4	193.0501
6		Dimethoxycinnamic	Acid	diMeCiA	C11H11O4	207.0657
7		Hydroxyferulic	Acid	HyF	C10H9O5	209.0455
8		Sinapic	Acid	SiA	C11H11O5	223.0612
9	Monoesters	p-Coumaroylquinic	Acid	pCoQA	C16H17O8	337.0929
10		Caffeoylquinic	Acid	CQA	C16H17O9	353.0878
11		Dehydrocaffeoylquinic	Acid	CQA-H_2O	C16H15O8	335.0772
12		Feruloylquinic	Acid	FQA	C17H19O9	367.1035
13		Dehydroferoloylquinic	Acid	FQA-H_2O	C17H17O8	349.0929
14		Dimethoxycinnamoylquinic	Acid	diMeCiQA	C18H21O9	381.1191
15		Hydroxyferuloylquinic	Acid	HyFQA	C17H19O10	383.0983
16		Sinapoylquinic	Acid	SiQA	C18H21O10	397.1140
17	Diesters	Di-p-coumaroyl-quinic	Acid	dipCoQA	C25H23O10	483.1296
18		Caffeoyl-p-coumaroylquinic	Acid	CpCoQA	C25H23O11	499.1246
19		Feruloyl-p-coumaroylquinic	Acid	FpCoQA	C26H25O11	513.1402
20		Dicaffeoylquinic	Acid	diCQA	C25H23O12	515.1195
21		Dimethoxycinnamoyl-p-coumaroylquinic	Acid	diMeCipCoQA	C27H27O11	527.1559
22		Hydroxyferuloyl-p-coumaroylquinic	Acid	HyFpCoQA	C26H25O12	529.1351
23		Caffeoyl-feruloylquinic	Acid	CFQA	C26H26O12	529.1351
24		p-Coumaroyl-sinapoylquinic	Acid	pCoSiQA	C27H27O12	543.1508

25		Caffeoyl-dimethoxycinnamoylquinic	Acid	CdiMeCiQA	C27H27O12	543.1508
26		Diferuloylquinic	Acid	diFQA	C27H27O12	543.1508
27		Caffeoyl-hydroxyferuloylquinic	Acid	CHyFQA	C26H25O13	545.1300
28		Dimethoxycinnamoyl-feruloylquinic	Acid	diMeCiFQA	C28H29O12	557.1664
29		Caffeoyl-sinapoylquinic	Acid	CSiQA	C27H27O13	559.1457
30		Hydroxyferuloyl-feruloylquinic	Acid	HyFFQA	C27H27O13	559.1457
31		Di-dimethoxycinnamoylquinic	Acid	didiMeCiQA	C29H31O12	571.1821
32		Feruloyl-sinapoylquinic	Acid	FSiQA	C28H29O13	573.1613
33		Dimethoxycinnamoyl-hydroxyferuloylquinic	Acid	diMeCiHyFQA	C28H29O13	573.1613
34		Dihydroxyferuloylquinic	Acid	diHyFQA	C27H27O14	575.1406
35		Dimethoxycinnamoyl-sinapoylquinic	Acid	diMeCiSiQA	C29H31O13	587.1770
36		Hydroxyferuloyl-sinapoylquinic	Acid	diHyFSiQA	C28H29O14	589.1563
37		Disinapoylquinic	Acid	diSiQA	C29H31O14	603.1719
38	Triesters	Tri-p-coumaroyl-quinic	Acid	tripCoQA	C34H29O12	629.1664
39		Di-p-coumaroyl-caffeoylquinic	Acid	dipCoCQA	C34H29O13	645.1613
40		Di-p-coumaroyl-feruloylquinic	Acid	dipCoFQA	C35H31O13	659.1770
41		Dicaffeoyl-p-coumaroylquinic	Acid	diCpCoQA	C34H29O14	661.1563
42		Dimethoxycinnamoyl-di-p-coumaroylquinic	Acid	diMeCidipCoQA	C36H33O13	673.1926
43		Di-p-coumaroyl-hydroxyferuloylquinic	Acid	dipCoHyFQA	C35H31O14	675.1719
44		Caffeoyl-feruloyl-p-coumaroylquinic	Acid	CFpCoQA	C35H31O14	675.1719
45		Tricaffeoylquinic	Acid	triCQA	C34H29O15	677.1512
46		Di-p-coumaroyl-sinapoylquinic	Acid	dipCoSiQA	C36H33O14	689.1876
47		Caffeoyl-dimethoxycinnamoyl-p-coumaroylquinic	Acid	CdiMeCipCoQA	C36H33O14	689.1876
48		Diferuloyl-p-coumaroylquinic	Acid	diFpCoQA	C36H33O14	689.1876
49		Caffeoyl-hydroxyferuloyl-p-coumaroylquinic	Acid	CHyFpCoQA	C35H31O15	691.1668
50		Dicaffeoyl-feruloylquinic	Acid	diCFQA	C35H31O15	691.1668
51		Dimethoxycinnamoyl-feruloyl-p-coumaroylquinic	Acid	diMeCiFpCoQA	C37H35O14	703.2032

(continued)

Table 3 (continued)

No.	Type	Name and abbreviation			Formula	$[M-H]^-$
52		Caffeoyl-p-coumaroyl-sinapoylquinic	Acid	CpCoSiQA	C36H33O15	705.1825
53		Feruloyl-hydroxyferuloyl-p-coumaroylquinic	Acid	FHyFpCoQA	C36H33O15	705.1825
54		Dicaffeoyl-dimethoxycinnamoylquinic	Acid	diCdiMeCiQA	C36H33O15	705.1825
55		Caffeoyl-diferuloylquinic	Acid	CdiFQA	C36H33O15	705.1825
56		Dicaffeoyl-hydroxyferuloylquinic	Acid	diCHyFQA	C35H31O16	707.1617
57		Di-dimethoxycinnamoyl-p-coumaroylquinic	Acid	didiMeCipCoQA	C38H37O14	717.2189
58		Feruloyl-p-coumaroyl-sinapoylquinic	Acid	FpCoSiQA	C37H35O15	719.1981
59		Dimethoxycinnamoyl-hydroxyferuloyl-p-coumaroylquinic	Acid	diMeCiHyFpCoQA	C37H35O15	719.1981
60		Caffeoyl-dimethoxycinnamoyl-feruloylquinic	Acid	diMeCiFQA	C37H35O15	719.1981
61		Triferuloylquinic	Acid	triFQA	C37H35O15	719.1981
62		Dihydroxyferuloyl-p-coumaroylquinic	Acid	diHyFpCoQA	C36H33O16	721.1774
63		Dicaffeoyl-sinapoylquinic	Acid	diCSiQA	C36H33O16	721.1774
64		Caffeoyl-feruloyl-hydroxyferuloylquinic	Acid	CFHyFQA	C36H33O16	721.1774
65		Dimethoxycinnamoyl-p-coumaroyl-sinapoylquinic	Acid	diMeCipCoSiQA	C38H37O15	733.2138
66		Caffeoyl-di-dimethoxycinnamoylquinic	Acid	CdidiMeCiQA	C38H37O15	733.2138
67		Diferuloyl-dimethoxycinnamoylquinic	Acid	diFdiMeCiQA	C38H37O15	733.2138
68		Hydroxyferuloyl-p-coumaroyl-sinapoylquinic	Acid	HyFpCoSiQA	C37H35O16	735.1930
69		Caffeoyl-feruloyl-sinapoylquinic	Acid	CFSiQA	C37H35O16	735.1930
70		Caffeoyl-dimethoxycinnamoyl-hydroxyferuloylquinic	Acid	CdiMeCiHyFQA	C37H35O16	735.1930
71		Diferuloyl-hydroxyferuloylquinic	Acid	diFHyFQA	C37H35O16	735.1930

72		Caffeoyl-dihydroxyferuloylquinic	Acid	CdiHyFQA	C36H33O17	737.1723
73		Di-dimethoxycinnamoyl-feruloylquinic	Acid	didiMeCiFQA	C39H39O15	747.2294
74		Disinapoyl-p-coumaroylquinic	Acid	diSipCoQA	C38H37O16	749.2087
75		Caffeoyl-dimethoxycinnamoyl-sinapoylquinic	Acid	CdiMeCiSiQA	C38H37O16	749.2087
76		Diferuloyl-sinapoylquinic	Acid	diFSiQA	C38H37O16	749.2087
77		Dimethoxycinnamoyl-feruloyl-hydroxyferuloylquinic	Acid	diMeCiFHyFQA	C38H37O16	749.2087
78		Caffeoyl-hydroxyferuloyl-sinapoylquinic	Acid	CHyFSiQA	C37H35O17	751.1879
79		Feruloyl-dihydroxyferuloylquinic	Acid	FdiHyFQA	C37H35O17	751.1879
80		Tri-dimethoxycinnamoylquinic	Acid	tridiMeCiQA	C40H41O15	761.2451
81		Dimethoxycinnamoyl-feruloyl-sinapoylquinic	Acid	diMeCiFSiQA	C39H39O16	763.2243
82		Di-dimethoxycinnamoyl-hydroxyferuloylquinic	Acid	didiMeCiHyFQA	C39H39O16	763.2243
83		Caffeoyl-disinapoylquinic	Acid	CdiSiQA	C38H37O17	765.2036
84		Feruloyl-dihydroxyferuloyl-sinapoylquinic	Acid	FHyFSiQA	C38H37O17	765.2036
85		Dimethoxycinnamoyl-dihydroxyferuloylquinic	Acid	diMeCidiHyFQA	C38H37O17	765.2036
86		Trihydroxyferuloylquinic	Acid	triHyFQA	C37H35O18	767.1829
87		Di-dimethoxycinnamoyl-sinapoylquinic	Acid	didiMeCiSiQA	C40H41O16	777.2400
88		Feruloyl-disinapoylquinic	Acid	FdiSiQA	C39H39O17	779.2192
89		Dimethoxycinnamoyl-hydroxyferuloyl-sinapoylquinic	Acid	diMeCiHyFSiQA	C39H39O17	779.2192
90		Dihydroxyferuloyl-sinapoylquinic	Acid	diFSiQA	C38H37O18	781.1985
91		Dimethoxycinnamoyl-disinapoylquinic	Acid	diMeCiHyFSiQA	C40H41O17	793.2349
92		Hydroxyferuloyl-disinapoylquinic	Acid	HyFdiSiQA	C39H39O18	795.2142
93		Trisinapoylquinic	Acid	triSiQA	C40H41O18	809.2298

Table 4 Selected dietary (vegetable/beverages) sources of CQAs – based on Clifford et al. (2017) and references cited in the main text (content given as per dried mass unless indicated otherwise)

Family	Species	Organs consumed	Identified compounds	Content
Aquifoliaceae	Yerba maté – *Ilex paraguariensis*	Leaves (tea)	CQAs and diCQAs in similar proportions	92 g/kg total CQAs
Asteraceae	*Arctium* sp. (burdock)	Tap roots	Over 30 CQAs and diCQAs	Exceeding 15 g/kg
	Cynara scolymus/ cardunculus – Globe artichokes and cardoons	Flower heads/ leaves	Over 30 various CQA isomers, 5-CQAs, and 1,5-diCQAs dominate depending on the season and variety	Flower heads – Over 5 g/kg
	Cichorium endivia and *C. intybus* – Endive – Various forms	Vegetative parts – Whole vegetable	3-CQA, dominant diCTA (chicoric acid – Dicaffeoyltartaric)	Up to 1 g/kg
	Lactuca ssp. – Lettuces	Whole vegetable	3-CQA	Up to 25 g/kg depending on variety and conditions
	Scorzonera hispanica – Black salsify	Tap roots	5-CQA and 3,5-*O*-diCQA dominating, several other diCQAs	Up to 130 g/kg
	Tragopogon porrifolius – Salsify	Tap roots		
	Stevia rebaudiana	Leaves	Several CQA and diCQA isomers – 4-CQA dominating	70 mg/kg
	Helianthus annuus – Sunflower	Seeds or fruits – Achenes	5-CQA dominating, followed by 3-CQA, 3,5-diCQA, 4,5-diCQA, 4-CQA, and several minor compounds of this class	Up to 30 g/kg
Convolvulaceae	*Ipomoea batatas* – Sweet potatoes	Tubers	5-CQA and 3,5-diCQA predominating	2 g/kg
Ericaceae	*Vaccinium myrtillus* – Bilberry	Fruits (berries)	5-CQA	500 mg/kg fresh fruit
Moraceae	*Ficus carica* – Figs	Fruits	5-CQA dominant	Up to 5.8 g/kg (peel)

(continued)

Table 4 (continued)

Family	Species	Organs consumed	Identified compounds	Content
	Jackfruit – *Artocarpus heterophyllus*	Fruits	5-CQA (dominant) and 4-CQA	Up to 14 g/kg
	Mulberry – *Morus alba*	Fruits	5-CQA (dominant)	500 mg/kg fresh fruit
Poaceae	Rice – *Oryza* sp.	Grain	3-CQA	10–20 mg/kg
Rosaceae	*Prunus armeniaca* – Apricot	Fruits/ kernels	3-CQA and/or 5-CQA dominant + several other in fruits	Few hundred mg/kg
	Prunus domestica – Plums and prunes	Fruits	5-CQA dominant, several other present	Total CQAs over 4 g/kg in prunes depending on variety
	Cydonia oblonga – Quince	Fruits	5-CQA dominant	Up to 3.2 g/kg
Rubiaceae	*Coffea arabica*, *C. canephora* – Arabica and robusta coffee	Grain (dehulled fruits)	5-CQA major compounds, dozens of other forms, including CQA and diCQA	Total CQa can exceed 100 g/kg
Solanaceae	*Solanum tuberosum* – Potato	Tubers	5-CQA major compound	Up to 6 g/kg, depending on variety and conditions
	S. melongena – Eggplant	Fruits	Several CQAs and diCQAs	3-CQA 0.4–3.0 g/kg, significant increase in grilled or baked fruit
	S. lycopersicon – Tomato	Fruits	3-CQA, 5-CQA and some diCQAs; content very variable; increases in grilled eggplant, tomato paste, etc.; but results are inconsistent (sometimes decreases)	Up to 1.5 g/kg

22.4 Metabolism and Bioavailability

Given the fact that phenolic acids are rarely present in foods in free forms, the evaluation of systemic availability of potential bioactive plant constituents is a major prerequisite for the interpretation of in vitro pharmacological testing.

Absorption of orally administered chlorogenic acid (5-*O*-caffeoylquinic acid) in rats was studied to obtain plasma pharmacokinetic profiles of their metabolites. Merely traces of metabolites, supposedly caffeic acid conjugates, caffeic acid glucuronides, caffeic acid sulfates/glucuronides, and ferulic acid sulfates/glucuronides, for 6 h after chlorogenic acid administration were observed. These metabolites reached their maximum concentrations of 0.12–0.34 μM within the first 0.5–1 h after administration; then their plasma levels decreased. However, no chlorogenic acid and its conjugates were detected. On the other hand, almost the whole amount of ingested chlorogenic acid was found in the small intestine for 6 h after oral administration. These results suggest that the intestinal mucosa or intestinal microflora may not possess esterase activity that would be able to cleave chlorogenic acid into caffeic and quinic acids which would decrease absorption of chlorogenic acid from the digestive tract (Azuma et al. 2000). Previous data from the literature also showed in an in vitro model using the small intestine from rats that only very little absorption of chlorogenic acid (0.1%) occurred (Spencer et al. 1999). Moreover, a study reported no evidence of enzymatic hydrolysis of chlorogenic acid by the intestine, liver, or human plasma but revealed that chlorogenic acid ingested by humans is most likely cleaved into caffeic acid and quinic acid by an esterase enzyme provided by the colonic microflora (Plumb et al. 1999). Strong evidence exists that the majority of chlorogenic acids are not absorbed in the proximal part of the gastrointestinal tract, unless transformed to caffeic and ferulic acids before being absorbed (Lafay et al. 2006). Generally, esterification of caffeic acid with quinic acid, as in chlorogenic acid, markedly reduced its absorption like the other esters. In fact, urinary excretion of chlorogenic acid represented 0.3% after a clinical trial with ileostomized patients. Other data showed that the metabolite concentration of chlorogenic acids was 100 times lower than that obtained with caffeic acid in the same conditions (Lafay and Gil-izguierdo 2008).

On the other hand, a few further studies providing data on CQAs metabolites in human plasma failed to detect other compounds than either conjugated or free hydroxycinnamic acids (DuPont et al. 2002; Nardini et al. 2002). Caffeoylquinic acids were detected in human plasma and urine only in form of conjugated and nonconjugated hydroxycinnamates such as caffeic acid, its methylated derivates ferulic acid and isoferulic acid, and the hydrogenation products dihydrocaffeic acid and dihydroferulic acid (Wittemer et al. 2005).

By contrast, a study on the absorption in humans of phenolic acids from coffee, a common beverage lacking free phenolic acids but particularly rich in bound phenolic acids, predominantly the mono-CQAs, indicates that hydrolysis of chlorogenic acid occurs early in the gastrointestinal tract. At 1 h after coffee brew consumption (actual intake of chlorogenic acids), a significant increase (91.1 $\pm$ 33.2 ng/mL) in free caffeic acid levels was found in plasma samples compared to time 0 (20 $\pm$ 10 ng/mL) (Nardini et al. 2002). The data clearly suggest that due to the absence of free caffeic acids in coffee brew used in the experiment, the level of plasma caffeic acid detected in the study represents the amount of caffeic acid absorbed following the in vivo release of free caffeic acid from bound complexes. Chlorogenic acid from administered coffee is not absorbed as such, but it undergoes hydrolysis in the

gastrointestinal tract by the action of cytosolic esterases in the gut mucosa or gut microflora. The study suggests that caffeic acid, even if present in the diet in bound forms, such as chlorogenic acids, is still bioavailable to humans.

However, Monteiro et al. (2007) found chlorogenic acids in human plasma samples after coffee consumption suggesting that the rapid transit through the stomach was not a critical point. The three main chlorogenic acids (3-caffeoylquinic, 4-caffeoylquinic, and 5-caffeoylquinic acids) and three dicaffeoylquinic acids were identified in plasma. Maximum concentration of total CGA reached 7.66 ± 2.5 μmol/l. Two plasma concentration peaks were observed, the first at 0.5–1.0 h and the second at 1.5–4.0 h after coffee consumption.

Studies on intestinal absorption and metabolism of chlorogenic acid revealed that around one-third of ingested chlorogenic acids in foods are absorbed and enter the bloodstream from the small intestine, with a possible contribution from the stomach; in healthy subjects with a functioning colon, the remaining two-thirds reach the large intestine (Stalmach et al. 2010). Experiments conducted in a rat model showed that chlorogenic acids are not hydrolyzed in the stomach but partially absorbed in an intact form; this could explain the early detection thereof in plasma, within 30 min after coffee consumption (Lafay et al. 2006).

Chlorogenic acid in parent form has been identified in human urine and plasma by several authors after ingestion of coffee; however, other authors failed to detect this phenolic acid in plasma or urine after the same consumptions. Farah et al. (2008) studied the pharmacokinetic profile and bioavailability of caffeoylquinic acids in healthy human subjects and found that the apparent bioavailability of them from a green coffee extract was approximately 33%. Urine was not the major route for excretion of caffeoylquinic acids in the human trial, but the smaller metabolites recovered suggested that the metabolism and excretion of esters, which influence the overall elimination kinetics in humans, could be quite variable and related to genetic polymorphisms.

To sum up, experiments that have been in place until now, carried out on humans and animals, have demonstrated that chlorogenic acids are bioavailable at physiological level and the variety of phenolic acids as circulating forms was very numerous thanks to the absorption as such and following metabolization and the occurrence of the microflora phenolic acid metabolites (Lafay and Gil-izguierdo 2008). During absorption, caffeoylquinic acids are conjugated in the small intestine, and later in the liver, where methylation, sulfation, and glucuronidation, as the main conjugation reactions, take place. This is a very important process not only in detoxification, to avoid any potential toxic effects, but also in eliminating them via the biliary or the urinary route by increasing their hydrophilicity (Heleno et al. 2015).

22.5 Bioactivities In Vitro (Cell Lines) and In Vivo (Animal Studies)

Despite of the large amount of data concerning in vitro bioactivity of phenolic acids, very little is known about the phenolic acid's bioactive forms in vivo and the mechanisms by which they may contribute toward disease prevention. Similarly,

only a few studies deal with the bioactive properties of their metabolites (Piazzon et al. 2012; Rechner et al. 2002), especially as most of those molecules are not commercially available. After absorption from the gastrointestinal tract, these molecules suffer conjugation reactions causing several changes in their initial structure and circulate in human plasma in their conjugated forms, such as glucuronide and methylated and sulfated derivatives. These changes in their structures may increase or decrease the bioactivity of the initial phenolic acids. Moreover, several studies dealing with the biological effects of phenolic acids have ignored the question of their achievable concentrations in the circulation after ingestion as well as the possibility of metabolism. As we have known from bioavailability study, caffeoylquinic acids, which are naturally esterified, are not or in little amount cleaved in the gastric lumen or the small intestine, but mostly they are cleaved in the colon by esterase activity of the gut microflora. Therefore, the urgent issue to be estimated is whether the identified conjugates and metabolites are physiologically relevant or can only be regarded as biomarkers of a phenolic acid-rich diet. So, detailed knowledge concerning the conjugative and metabolic events and resulting plasma levels following the ingestion of a hydroxycinnamates-rich diet is crucial for understanding their bioactivity.

22.5.1 Antioxidant and Antimutagenic Activity In Vitro

Several experimental and epidemiological studies suggested that chlorogenic acids of daily diet contributed to the protection against various degenerative diseases (Liang and Kitts 2016). Their effects on health have been more particularly attributed to their antioxidant properties. The underlying mechanism(s) for specific health benefits attributed to chlorogenic acids involve(s) mitigating oxidative stress and hence the related adverse effects associated with an unbalanced intracellular redox state.

The base of antioxidant action of chlorogenic acids (CGAs) is their ability to donate hydrogen atoms to reduce free radicals and to inhibit oxidation reactions. After donating hydrogen atoms, chlorogenic acids are oxidized to their respective phenoxyl radicals, and these phenoxyl radicals are quickly stabilized by resonance stabilization (Liang and Kitts 2016). The chlorogenic acid is an excellent natural scavenger among many polyphenol compounds, because the one electron oxidation product of CGA formed by reaction with free radicals is rapidly broken down to further products that cannot generate any free radicals (Shibata et al. 1999).

Although a majority of isomers of chlorogenic acid – such as caffeoylquinic acids (CQAs): 3-CQA, 4-CQA, 5-CQA, 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA – are potent antioxidants (Xu et al. 2012), chemical-based or cell-based assays directed at characterization of the antioxidant capacity have been focused mostly on the 3-CQA isomer (chlorogenic acid). Chlorogenic acids react with different sources of free radicals at different rate constants; they have the capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, superoxide anions ($^{\cdot}O_2^-$), hydroxyl radicals ($^{\cdot}OH$), 2,21-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot}$) radicals,

lipid oxidation, and peroxynitrite ($ONOO^-$). The efficiency of 5-CQA isomer reacting with free radicals such as superoxide, hydroxyl radical, peroxyl radical, and peroxynitrite is species-specific, as has been determined by the second-order rate constants $0.96 \pm 0.01 \times 10^6$ $M^{-1}s^{-1}$, $3.34 \pm 0.19 \times 10^9$ $M^{-1}s^{-1}$, $1.28 \pm 0.11 \times 10^5$ $M^{-1}s^{-1}$, and $1.6 \pm 0.7 \times 10^5$ $M^{-1}s^{-1}$, respectively (Liang and Kitts 2016).

This antiradical activity of chlorogenic acids has been attributed to important action such as DNA-protective mechanism against the radical damage caused to DNA, including DNA strand breakage. Six CGA isomers, including three caffeoylquinic acid isomers 3-CQA, 4-CQA, and 5-CQA and three dicaffeoylquinic acid isomers 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA, were shown to exhibit a protective effect against DNA plasmid chromosome breaks induced by H_2O_2. Dicaffeoylquinic acids demonstrated better protective antioxidant activity than caffeoylquinic acids against the radical damage caused to DNA, mostly because they have more hydroxyl groups (Xu et al. 2012).

On the other hand, the redox cycling of chlorogenic acids in the presence of oxygen and transition metals takes place to form reactive oxygen species that are capable to damage macromolecules, such as DNA and lipids (Sakihama et al. 2002). So, in systems containing redox-active metals such as Cu and Fe, prooxidant activity of hydroxycinnamates occurred. Chlorogenic acids are relatively weaker pro-oxidants compared to caffeic acid; the reason for this difference is that caffeic acid is a simpler p-coumaric derivative, where CGA is esterified and contains a quinic-carbohydrate moiety. Moreover, CGAs in the presence of Cu ions are relatively short-lived radicals; nevertheless they can result in a prooxidant effect, such as DNA single-strand breaks (Liang and Kitts 2016).

Many examples of antioxidant properties of chlorogenic acids, attributed to prevention of crucial biomolecules, have been shown in vitro on **chemical-based assays**. The research by Shibata et al. (1999) studied protective effect of CGA against detrimental activity of hypochlorous acid (HOCl), a secondary byproduct of oxidation of chloride by H_2O_2, which initiate a mutagenic response as a reaction with amines to produce chloramine (NH_2Cl). Chloramine is an oxidant and potent mutagen. CGAs, specifically 5-CQA, have been able to protect against NH_2Cl-induced DNA breakage due to its good ability to scavenge HOCl and other intermediates of oxygen. Additionally, the antioxidant reaction occurs more rapid in acidic than neutral pH; thus CGAs from diet could be excellent protectors from *H. pylori*-induced gastric mucosal lesions, partially caused by intermediates of oxygen produced by activated neutrophils (Shibata et al. 1999). The anti-peroxidative activity of CGA showed experimental study where the protected substrate was LDL. Protection against LDL oxidation is crucial for prevention of the initial step in the development of many disorders such as atherosclerosis. Studies based on incubating isolated LDL with oxidizing agents in vitro showed that 3-CQA was effective at mitigating both copper-induced LDL oxidation and ferryl myoglobin-induced LDL oxidation (Liang and Kitts 2016). These results correspond to other studies in rat brain in vitro that reported reduced malondialdehyde (MDA) content by chlorogenic acid. The mechanisms through CGA exert its neuroprotective

properties were inhibiting AChE and BChE activities as well as preventing oxidative stress-induced neurodegeneration (Oboh et al. 2013).

22.5.2 Cell Lines and In Vivo Studies

Further type of experiments focused on characterization of the antioxidant capacity of CGAs used **cell-based models**. The oxidative stress was stimulated by various oxidative stressors (e.g., t-BHP, H_2O_2, $FeSO_4$) in PC12 cells, and for its evaluation diverse biomarkers such as the reduction of lipid peroxidation product, the extent of ROS formation, and GSH depletion were used. Genomic DNA integrity is also an important biomarker of redox status because reactive species continuously attack DNA structure and cause different kind of DNA lesions. The results showed the utility and effectiveness of CGA isomers to mitigate intracellular oxidative stress (Liang and Kitts 2016).

3-CQA reduced the DNA oxidative damage caused by X-ray irradiation by 4.49%~48.15%. In addition to this direct effect, this compound reduced UV photo-lesions in human blood lymphocytes, mouse epidermal cell line or human HaCaT keratinocytes (Cha et al. 2014; Feng et al. 2005).

The **animal-based assays** have confirmed antioxidant activity of CGAs based on evidence from in vitro chemical and cellular-based assays. Observations from animal experimental models examined the redox status after exposition thereof to a variety of forms of oxidative stress when animals were pretreated with 3-CQA. In diabetic rats feeding 3-CQA, it was observed effectively reduced lipid hydroperoxide production and increased level of non-enzymatic antioxidants such as reduced glutathione and vitamins C and E. This ability of CGAs is attributed to prevention of environmental toxicity, for instance, caused by heavy metal pollution. Pretreatment of rats with 3-CQA before Cd exposure significantly restored the depleted levels of GSH, vitamin C, and vitamin E and attenuated harmful MDA levels in brain tissue (Liang and Kitts 2016). Other studies have shown that affinity of 3-CQA to reduce lipid peroxidation and free radical scavenging activity can alleviate the oxidative stress induced by methamphetamine or scopolamine and prevent the accumulation of MDA in the central nervous system (Liang and Kitts 2016). The 3-CQA also protects against oxidative stress through its activation of Nrf2 nuclear translocation and upregulation of cellular antioxidant enzymes, suppression of ROS-mediated NF-κB, AP-1, and MAPK activation. These results could be attributed to chemopreventive effects of chlorogenic acid and its protection against environmental carcinogen-induced carcinogenesis (Feng et al. 2005).

CQAs are able to attenuate harmful impact on intracellular redox balance caused not only by chemical but also by physical factors, such as UV or γ-irradiation. Topical delivery of 3-CQA in guinea pigs during exposure to UVB reduced photo-oxidation-induced damage of skin. Moreover oral administration of 3-CQA to mice at a concentration of 100 mg/kg body weight before exposure to gamma radiation significantly reduced chromosomal damage (Liang and Kitts 2016).

In conclusion, there is strong evidence that CGAs are effective antioxidants that will protect against excessive oxidation reactions in vivo by upregulating redox-related nuclear transcription factors involved in the expression of antioxidant enzymes (Magielse et al. 2014).

22.5.3 Anti-Inflammatory Activity

Similarly to other bioactivities, anti-inflammatory properties of caffeoylquinic conjugates are represented mostly by the chlorogenic acid (3-CQA) studies. Also, similarly to antioxidant properties, anti-inflammatory actions are thought to be a basis for broader therapeutic/preventive effects. Inflammation processes when occur chronically or excessively may lead to developing of lifestyle diseases and complications or even severe malignancies. Chlorogenic acids target multiple signaling pathways involved in generating inflammatory response on different levels, such as NFκB pathway inhibition, scavenging ROS, and direct inhibition of enzymes such as COX, NOS, and LOX, as well as normalizing the levels of circulating cytokines and balancing the associated gene expression (Clifford et al. 2017; Naveed et al. 2018; Yamagata 2018).

For example, suppressing of Toll-like receptor in Con-A challenged mice leads to amelioration of hepatitis (Yuan et al. 2017). So, the effects are indeed pleiotropic and cannot be dissected from other pathophysiological processes, such as dyslipidemia and other symptoms of metabolic syndrome.

Several recent reviews on the pharmacology of chlorogenic acids unanimously conclude that anti-inflammatory properties are well-documented in vivo (Clifford et al. 2017; Santana-Gálvez et al. 2017; Naveed et al. 2018; Yamagata 2018). These properties result in protection against neuroinflammatory injury, damage of the vascular system by reducing hypertension and atherosclerosis, damage of hepatocytes and other tissues in the GI tract, against promotion of tumors, and finally in reducing inflammation-related pain.

22.6 Disease Prevention

Recent basic and clinical research studies have mentioned about reduction in the risk of a variety of diseases following CQA consumption. It is postulated that CQAs are able to exert significant roles in prevention development of many diseases as cardiovascular diseases, type 2 diabetes mellitus, obesity, cancers, hepatic steatosis, stroke, and Alzheimer's disease.

22.6.1 Cardioprotective Properties

Cardiovascular diseases (CVDs) continue to be the leading cause of morbidity and mortality in the industrialized world and account for 15.2 million deaths worldwide

in 2016 (WHO 2018). The molecular mechanisms underlying CVD are closely linked to cellular oxidative stress and inflammation, which contribute to LDL oxidation, endothelial dysfunction, atherosclerotic plaque formation, plaque rupture, vascular remodeling, and atherothrombosis (Pashkow 2011; Hajjar and Gotto 2013; Hussain et al. 2016).

Natural products containing CQAs could play an important role in regulation of ROS and glutathione level in the cells. Clinical evidences have shown an association between GSH level and CVD. Low GSH level was observed in patients with the most severe cases of heart failure, and patients with cardiac diseases showed 21% depletion in blood GSH than healthy controls (Damy et al. 2009). 3-*O*-caffeoylquinic acid (CGA) has been shown to induce glutathione-linked enzymes via Nrf2 signaling pathway; it increases the transcriptional expression of γ-GCL, HO-1, and GSTA1 (Boettler et al. 2011).

Recent clinical studies have shown that artichoke (*Cynara scolymus*) extract containing chlorogenic acid, 1,5- and 3,4-di-*O*-caffeoylquinic acid, and cynarin as the predominant polyphenolic compounds among hydroxycinnamates and apigenin and luteolin, and their glycosides as the main flavonoids, may favor CVD prevention by acting on different factors, which is increasing HDL-C as well as decreasing LDL-C, triglycerides, and total cholesterol (Rondanelli et al. 2013; Rangboo et al. 2016; Bundy et al. 2008; Lupattelli et al. 2004; Englisch et al. 2000). Not all clinical trials showed consistent results which may be elucidated by a slightly different methodology and different purification and standardization, as well as the stability of the supplements consisting artichoke extracts. However, in a report about artichoke leaf extract (ALE) for treating hypercholesterolemia created by Cochrane Database of Systematic Reviews, which is the leading journal and database for systematic reviews in health care, authors concluded that there is an indication that ALE has potential in lowering cholesterol levels, but the evidence is, as yet, not convincing (Wider et al. 2013). One of the most likely explanation of ALE impact on HDL cholesterol increase is associated with chlorogenic acid which protects paraoxonase-1 (PON-1) activity in HDL-C, which was demonstrated in in vitro study (Gugliucci and Bastos 2009). Paraoxonase 1 (PON-1) is a high-density lipoprotein (HDL)-associated serum enzyme, and it has been shown to protect against CVD, by preventing the formation of oxidized HDLs and low-density lipoproteins (LDLs) (Aviram et al. 1998); by hydrolyzing the thiolactone form of homocysteine, which alters proteins in the arterial wall (Jakubowski 2000); and by hydrolyzing platelet-activating factor, a bioactive phospholipid which is involved in vascular disease development (Rodrigo et al. 2001). Decreasing total cholesterol and LDL-C was observed also in other studies carried out with ALEs in animal models (Küskü-Kiraz et al. 2010; Qiang et al. 2012).

Some of the studies attribute luteolin impact on decreasing total cholesterol and LDL-C by inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, which is a key enzyme in the liver cholesterol biosynthesis pathway (Gebhardt 1996, 2002). Even though cynarin did not show inhibition of HMG-CoA reductase activity in the abovementioned studies, some older studies showed its effect on lowering plasma lipids (Montini et al. 1975; Kraft 1997;

Fintelmann and Menssen 1996; Lietti 1977; Wójcicki 1978; Thompson Coon and Eernst 2003). Impact of other CAs on lipid profile was also analyzed. In a 2002 study, Rodriguez de Sotillo and Hadley found that in obese, hyperlipidemic, and insulin-resistant Zucker rats which were infused with chlorogenic acid via intravenous infusion, fasting plasma cholesterol and triacylglycerol (TG) levels were significantly reduced, similar to liver triacylglycerol levels (Rodriguez de Sotillo and Hadley 2002). More recently, in addition to improve plasma lipid profiles, chlorogenic acid was shown to inhibit lipoprotein lipase (LPL) activity in vivo and elevate peroxisome proliferator-activated receptor-α (PPARα) expression in the liver (Li et al. 2009; Cho et al. 2010). In a 2014 in vivo study, Huang et al. (2014) by using real-time polymerase chain reaction revealed that chlorogenic acid altered the mRNA expression of the transcription factors peroxisome proliferator-activated receptor-α (PPARα) and liver X receptor-α (LXRα) and targeted genes involved in hepatic fatty acid uptake, β-oxidation, fatty acid synthesis, and cholesterol synthesis. They concluded that chlorogenic acid may improve lipid metabolism disorders by altering the expression of PPARα and LXRα. Taking into account recent evidence, it is believed that cholesterol-lowering effects of chlorogenic acid in SD rats are most likely mediated by increasing fatty acid utilization in the liver via the upregulation of PPARα mRNA (Wan et al. 2013).

22.6.2 Antidiabetic Activities

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by insulin resistance, pancreatic β-cell dysfunction, and hyperglycemia (Dada et al. 2017). Studies have shown contrary association between consumption of product containing CAs and lower risk of type 2 diabetes. For example, it is reported that there is reduction of risk of T2DM by 30% for those who drink 3–4 cups of decaffeinated coffee containing high contents of CAs (Huxley et al. 2009). In randomized crossover trial conducted on 15 overweight men, it was observed that chlorogenic acid and trigonelline ingestion significantly reduced early glucose and insulin responses during an OGTT (oral glucose tolerance test) (van Dijk et al. 2009). Several other human studies have been done to assess the antidiabetic activity of chlorogenic acid-rich foods and supplements or pure chlorogenic acid (Thom 2007; Ahrens and Thompson 2013; Iwai et al. 2012; Johnston et al. 2003). Some of them demonstrated that caffeinated and/or decaffeinated coffees (rich in chlorogenic acid) significantly decreased glucose-dependent insulinotropic polypeptide (GIP) and increased glucagon-like peptide 1 (GLP-1) secretion after consumption. Authors suggested that chlorogenic acid might have an antagonistic effect on glucose transport (Johnston et al. 2003). Another randomized, double-blind, placebo-controlled trial, performed in 55 overweight subjects with impaired fasting glycaemia, showed that supplemented group with extract from *Cynara scolymus* had significant decreases of FBG (fasting blood glucose), HOMA (homeostatic metabolic assessment), glycosylated hemoglobin, ADAG (A1c-derived average glucose), and lipidic pattern (Rondanelli et al. 2014). Furthermore, in vitro evidence demonstrated that

chlorogenic acid increased cell insulin secretion and insulin sensitivity (Tousch et al. 2008). By investigating the mechanisms of chlorogenic acid activity, we learn that it inhibited glucose-6-phosphate translocase 1 and reduced the sodium gradient-driven glucose transport in the intestine (McCarty 2005). What is more, it suppresses hepatic gluconeogenesis through the inhibition of glucose-6-phosphatase (G6P-ase) activity (Bassoli et al. 2008) and stimulates glucose uptake in myotubes (Prabhakar and Doble 2009) and adipocytes (Alonso-Castro et al. 2008). A study of Ong et al. (2013) demonstrated that chlorogenic acid regulates glucose and lipid metabolism via the activation of AMPK (5′-adenosine monophosphate-activated protein kinase). It inhibited hepatic G6P-ase expression and activity, attenuated hepatic steatosis, and improved lipid profiles and skeletal muscle glucose uptake, which in turn improved fasting glucose levels, glucose tolerance, insulin sensitivity, and dyslipidemia.

22.6.3 Hepatoprotective Activity

Several studies have shown the beneficial impacts of caffeoylquinic acids and products that contain them on liver disorders. One of them is nonalcoholic steatohepatitis (NASH), a multifactorial disorder, characterized by fatty infiltration in the liver and steatosis that may culminate in cirrhosis and hepatocellular carcinoma. Oxidative stress, hepatocellular inflammation, and insulin resistance play an important role in the pathogenesis of NASH. Rangboo et al. (2016) decided to carry out a randomized double-blind clinical trial in which patients suffering NASH were randomly assigned to receive *Cynara scolymus* extract or placebo for 2 months. Significant reduction of triglycerides and total cholesterol was observed, as well as a decrease in serum ALT and AST (effective biomarkers in the diagnosis of hepatic damage) in the group receiving the extract in comparison with the placebo group. The authors suggested that this effect could be attributed to the antioxidant ingredients in *Cynara scolymus* extract, in which chlorogenic acid is the most active antioxidant (Gebhardt 1998). Also, several in vitro and animal studies evaluated the antioxidative and free radical scavenging potential of *Cynara scolymus* extracts in the protection of hepatocytes from oxidative stress (Heidarian et al. 2013; Metwally et al. 2011; Mehmetçik et al. 2008; Miccadei et al. 2008). Moreover, four dicaffeoylquinic acid derivatives isolated from the water extract of propolis have been shown to be effective against carbon tetrachloride (CCL4) liver injury in an in vitro assay (Basnet et al. 1996). Wang et al. (2009) reported anti-hepatitis-B virus activity of chlorogenic acid, quinic acid, caffeic acid, and coffee rich in chlorogenic acid in vitro and in animal models. Recent study indicated that roasting levels of coffee correspond to total chlorogenic acid contents as well as antioxidant and anti-inflammatory activities (Jung et al. 2017). Data suggest that these activities are negatively correlated with roasting levels in the cell models. Total chlorogenic acid contents were higher in lightly roasted coffee extract than other roasted groups. In addition, lightly roasted coffee extract had the highest antioxidant activity. Intracellular glutathione (GSH) concentration and mRNA expression levels of genes related to GSH synthesis were negatively related to roasting levels.

22.6.4 Neuroprotective Activity

Studies have shown that excessive oxidative stress in the brain leads to accelerated aging and impacts the severity of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and autism spectrum disorder (ASD) (Frandsen and Narayanasamy 2018). Caffeoylquinic acids are known for their antioxidant activities (Clifford 1999; Cao et al. 2010), and their metabolites may impact on redox processes in the brain (de Paulis et al. 2002). Soumyanath et al. (2012) demonstrated that treatment with water extract of *Centella asiatica* improves learning and memory deficits in Tg2576 mice, an animal model of Aβ (amyloid-β) accumulation, where the accumulation of amyloid-β (Aβ) is a hallmark of Alzheimer's disease and is known to result in neurotoxicity. In the next study, researchers confirmed the neuroprotective effects of caffeoylquinic acids and that isochlorogenic acid A and 1,5-dicaffeoylquinic acid were the most active in protecting MC65 cells from Aβ-induced cell death (Gray et al. 2014). Each compound was also able to attenuate Aβ-induced alterations in tau expression and phosphorylation in both cell lines (Gray et al. 2014). Many studies have found that chlorogenic acid has the ability to attenuate oxidative stress in numerous neurological disorders (Kwon et al. 2010; Kim et al. 2012). Moreover, the recent study revealed that chlorogenic acid acts as a protective agent against glutamate-induced cortical neuron injury, where glutamate excitotoxicity has proven to play a key role in several neurodegenerative disorders, including Huntington's disease and multiple sclerosis, Alzheimer's disease, and Parkinson's disease, as well as other neurological disorders (Rebai et al. 2017). In the latest review article about cognitive and neuroprotective effects of chlorogenic acid, it was summarized that preclinical and clinical studies provide evidence that chlorogenic acid supplementation could protect against neurological degeneration and the resulting diseases associated with oxidative stress in the brain; however, no formal, well-controlled studies have been performed to date (Heitman and Ingram 2017). Also, in a second review from 2017 about chlorogenic acid and mental diseases, the authors concluded that chlorogenic acid possesses neuroprotective effects under both in vitro and in vivo models (Nabavi et al. 2017).

22.7 Chlorogenic Acids: Epidemiological Studies

There are no specific epidemiological studies concerning use of CGAs and morbidity on certain diseases. For this paper the epidemiological analysis was led by a comparison of CGA-rich nutrition product consumption with analysis of potential disorders within CGA activity. As CGAs mainly act in the gastrointestinal tract, especially on the liver, this organ was selected for further studies. The activity against colorectal cancer was also analyzed.

Liver disorders, according to WHO report, are at the forefront of most often cause of death in the world. These include hepatitis virus infection, mainly B and C, liver cancer, cirrhosis, and chronic liver diseases of different etiologies, among them excessive alcohol consumption or obesity. Other nonfatal liver disorders significantly

affect health and life quality and mostly depend on diet and lifestyle. This second class includes nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). NAFLD is defined as a presence of $\geq$5% of hepatic steatosis due to causes other than alcohol use, viral infections, or certain medicine intake. It is recognized as the liver component of a metabolic syndrome (MetS) often appearing in obesity (Younossi et al. 2016). NASH occurs when significant fibrosis is present due to inflammatory process that may lead to cirrhosis or liver cancer. It is estimated that about one of five people with NAFLD has NASH (Pimpin et al. 2018).

Simple fatty liver, as NAFLD is called, is associated with a high burden of metabolic comorbidities including obesity, type 2 diabetes, hyperlipidemia, hypertension, and MetS. NAFLD is the most common liver disease in the world affecting 25.24% of global population with the highest prevalence in the Middle East and South America and lowest in Africa (Younossi et al. 2016). Estimated prevalence of NAFLD in Europe is 23.71%, while NASH reaches up to 11.9% counted by liver transplantations for cirrhosis from 1988 to 2016 (ELTRA). Coexistence of diabetes or obesity is an important risk factor of NAFLD/NASH morbidity. It is known that for one unit of body mass index (BMI), risk of occurrence of NAFLD increases by 13–38%, while for 1 cm more in waist circumference, its change reaches 3–10%.

Chlorogenic acid is known for its antioxidant, anticarcinogenic, and anti-inflammatory properties. It is a common constituent present in human diet in products like coffee, artichokes, potatoes, berries, peach, apple, anise, fennel, etc. (Clifford 1999). Despite many sources, most of them cannot be considered in epidemiological studies, because the dosage is too low or consumption is occasional and difficult to determine. Seasonal availability of most products causes even more considerable fluctuations. Potato tubers, due to their year-round availability and wide consumption, might be an epidemiologically interesting source of CGAs reaching 17.3 mg/100 g of fresh weight, but the cooking and baking processes cause 60% and 100% of their loss, respectively (Dao and Friedman 1992).

Apples were the most produced fruit in EU in terms of quantity, with 12.7 million tons harvested in 2015 (or almost 25 kg per EU inhabitant) (Eurostat). Among other fruits apples have the highest amount of free phenolics which can be easily absorbed into the bloodstream. Pure 5-*O*-caffeoylquinic acid is recovered from the intestine reaching even 60.8% of intake (Borges et al. 2013). It is also the main phenolic acid found in apples, with the second highest antioxidant activity among all other compounds, except rutin which ranks first. 100 mL of apple juice contains about 3.5 mg of pure 5-*O*-caffeoylquinic acid (Borges et al. 2013), while that of the fruit and peel reaches 12.6 mg/100 g FW and 0.26 mg/g FW, respectively (Kalinowska et al. 2014). CGA concentration is not constant, and factors like cultivar type, maturity of the fruit, conditions of cultivation, rising, harvest, and storage significantly affect those values.

In turn artichokes provide about 450 mg/kg of chlorogenic acid and are rich source of cynarin, an isomer of 1,5-diCGA formed spontaneously in water and constituting about 800–1400 mg/kg dry mass of capitula (Clifford 1999). Global consumption of artichokes in 2015 reached 1,637 tons, and nearly one-third out of it was eaten in Italy (511,000 tons). That gave 8.55 kg per person per year which is

impressive compared with global average of 1.07 kg/year. In the USA according to USDA, Economic Research Service in 2017, artichoke consumption per capita was 1.43 pounds, equivalent to 0.648 kg per year. An average Italian eats about 23 g of artichokes per day compared with 1.8 g for an average American. However, the consumption trend is rising; this cannot be taken into epidemiological consideration, since the highest Italian consumer eats about 10 mg of CGA daily through artichokes (Parr et al. 2018).

Compare with a single espresso that can contain up to 116 mg of chlorogenic acid, potatoes are rather poor source of this polyphenol (Ludwig et al. 2014). Therefore, for further epidemiological studies, coffee was selected as a most valuable source of CGAs in a human diet.

Coffee is one of the most frequently consumed beverages in the world with about 500 billion coffee cups drank annually. In 2017/2018, the world's total coffee consumption was estimated to be 9.72 million tons, with almost one-third of which was in Europe. According to the International Coffee Organization (ICO) statistics, coffee consumption varies significantly across Europe. The highest coffee consumption is estimated in Scandinavian countries reaching annually 12 kg per person in Finland or 9.9 kg in Norway compared with 5.8 kg in Italy or 5.1 kg in France. In several countries like Denmark, the USA, Brazil, and Japan, coffee is the major source of dietary phenolic antioxidants (Komes and Belščak-Cvitanović 2014).

The CGA content in coffee varies depending on species and roasting, grinding, or brewing conditions (Perrone et al. 2010). In commercial coffee industry, two species are the most valuable: *Coffea arabica* with fine, mild aroma and *Coffea canephora* var. *robusta* with more distinctive taste. Generally arabica contains less CGAs than robusta and about 50–60% less of caffeine. The second main factor influencing CGAs content is roasting, which causes progressive destruction of CGAs. Coffee beans roasted in 220 °C for 6, 7, 8, 13, and 15 min lose about 25%, 60%, 70%, 98%, and 99% of CGAs, respectively (Antonio et al. 2010), which remain consistent with other papers (Ludwig et al. 2014; Farah et al. 2005; Priftis et al. 2018). Both of those components significantly influence the taste of coffee and can be seen in regional preferences. People from South European countries generally drink darker roasted coffee and blends with robusta varieties as a basis, while North European consumers favor mild arabicas and a lighter roasting (Ludwig et al. 2014).

Coffee drinking is very popular around the world, and many techniques of brewing are used, listing filter coffee, French press, instant, Italian, Turkish, or high-pressure espresso. Filter coffee provides the lowest content of beneficial polyphenolic compounds, while most was found in the instant one. By now 40% of instant coffee is made from robusta beans from *Coffea canephora*, which explains the higher content of CGAs compared with classical brewed coffee. Relatively high amount of chlorogenic acid is present in espresso coffee reaching 0.43–4.31 mg/mL which on average volume about 35 mL gives 15.05–150.85 mg per serving. Filter coffee consists 0.41–1.57 mg/mL making about 41–157 mg per 100 mL serving. In this case differences are caused by different times of brewing which directly affect the extraction process (Ludwig et al. 2012; Jeon et al. 2017). Grain grinding degree

affects the content of CGAs in accordance with the principle that the smaller the particles are taken, the more effective the extraction process is (Jeon et al. 2017). Reheating of coffee to boiling temperature elevates CGA and caffeine content on approximately 23% (Niseteo et al. 2012).

As the roasting and brewing time significantly changes CGA content in coffee, other coffee additives change the bioavailability of polyphenols. Addition of milk significantly decreases polyphenolic content and antioxidant capacity of coffee which may be caused by potential interactions between polyphenols and milk proteins (Niseteo et al. 2012). Interestingly Ludwig et al. did not confirm those findings in his study showing that CGA content in espresso and espresso-based latte or cappuccino is comparable (Ludwig et al. 2014). Duarte and Farah (2011) found that instant coffee dissolved in milk has about 19% less CGA metabolites compared to one dissolved in water and that addition of milk causes reduction of coffee metabolite amount in urine. Unfortunately, there is no additional data about "milk" used in all referred experiments.

Decaffeination process can be done by several methods based on organic solvents used or nonorganic-solvent-based processes. Dichloromethane or ethyl acetate is the most popular organic solvent used in decaffeination process. Other methods without organic solvent are based on water decaffeination or carbon dioxide caffeine extraction (Lack and Seidlitz 2012). In methods using organic solvent, some of chlorogenic acid is lost because of repeated heating process to evaporate the solvent. Lima et al. show that about 60% of chlorogenic acid is lost in decaffeination using dichloromethane in the case of green coffee, while in roasted coffee its content decreased only at about 5% compared with normal caffeinated coffee. Chlorogenic acid content for green coffee reached (g/100 g of ground coffee) 4.95 ± 0.043 and 1.95 ± 0.041 for caffeinated and decaffeinated, respectively, while for roasted coffee those values were 0.40 ± 0.017 and 0.38 ± 0.008 (Lima et al. 2013). In turn, when decaffeination is performed using a method without organic solvent like in water, coffee brews contain higher total content of chlorogenic acids than its regular coffee counterparts. Farah et al. (2005) report an average increase of about 18% in total CGAs in coffee decaffeinated with this technique. Green coffee steaming prior decaffeination is often used to moisten the bean and cause it to swell which makes subsequent caffeine extraction easier. Steaming causes about 10–20% loss of CGAs (Steinhart et al. 2001; Budryn et al. 2015). Most decaffeinated coffees available is decaffeinated using organic solvents. Coffees usually defined as "bio" are mostly decaffeinated with water.

Considering the above, determining the average amount of specific constituents is ineffective, considering the number of variables that appear within one "cup of coffee." Each cup of coffee consists relatively high amount of chlorogenic acid that possesses many health-promoting properties. Most of them are related to the treatment of metabolic syndrome including antioxidant, anti-inflammatory, antilipidemic, antidiabetic, and antihypertensive activities. Benefits of coffee drinking strictly overlap them suggesting that the range of coffee activities results from its high content of chlorogenic acid. However coffee is also the major natural dietary source of caffeine, a purine alkaloid, exerting stimulatory effect on sympathetic

nervous system. Kahweol and cafestol, the coffee diterpenes, are also reported to administer liver protective action (Muriel and Arauz 2010). Coffee drinking health benefits therefore cannot be linked just with CGAs, as every single cup possesses a number of active constituents which may act simultaneously or in contrary to polyphenols. Studies investigating the relationship between coffee consumption and different disease morbidities are of interest to many research groups. However, such studies are too often restricted to testing an influence of a 'cup of coffee' without quantitative analysis of active constituents.

Caffeine role in liver protection remains unclear although some efforts are taken to explain this issue. Caffeine-containing beverages including coffee, decaffeinated coffee, tea, chocolate, and Coca-Cola were investigated on the risk of symptomatic liver cirrhosis. No evidence of significant trends was observed in examined beverages except in coffee. In this case inverse association was noticeable with dose-dependent manner starting with one cup of coffee a day. Liver cirrhosis odds ratios decreased from 1.0 for control group of non-coffee drinkers to 0.47, 0.23, 0.21, and 0.16 in 1, 2, 3, 4, or more cups of coffee drinkers, respectively (Corrao et al. 2001). That remains consistent with Kennedy et al.'s findings who noticed the association between high coffee consumption and reduction of hepatocellular carcinoma risk. In this case in turn caffeinated and decaffeinated coffee were examined, both showing downward risk trend, but to a lesser extent for decaffeinated one (Kennedy et al. 2017). As decaffeinated coffee consists less CGAs, and no additive data determining "coffee" is provided, fluctuations between coffee brewing might occur causing significant differences in CGAs concentration. After all the abovementioned findings may suggest the positive role of constituents other than caffeine in hepatocellular carcinoma risk reduction.

Basically, most of the research confirm the finding that coffee drinking has a positive impact on liver enzymes. These phenomena were firstly observed in 1985, and since then numerous studies confirmed it. It may be a surprising fact that coffee drinking is generally linked with less healthy lifestyle including cigarette smoking, less physical activity, and less healthy diet. The reduction of transaminases (alanine transaminase (ALT), aspartate transaminase (AST)), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) is significant and dose dependent. In 1999–2010, in US National Health and Nutrition Examination Survey, 27,793 participants were examined for association of coffee drinking and level of enzyme activity. It was confirmed that drinking of three or more cups of coffee a day lowers the level of liver enzymes with OR = 0.75 (95% CI: 0.63–0.89), 0.82 (95% CI: 0.68–0.98), 0.73 (95% CI: 0.55–0.95), and 0.69 (95% CI: 0.57–0.83) for ALT, AST, APT, and GGT, respectively. The similar inverse association was observed also in case of decaffeinated coffee. Drinking of two or more decaffeinated cups of coffee a day compared with non-coffee drinkers had OR = 0.62 (95% CI: 0.41–0.94), 0.74 (95% CI: 0.49–1.11), and 0.70 (95% CI: 0.49–1.00) for ALT, AST, and GGT, respectively (Xiao et al. 2014). Serum levels of ALT and AST were also lower for coffee drinkers in dose-dependent manner in recent prospective cohort study of European Prospective Investigation into Cancer and Nutrition (EPIC). However, some of those observations were more significant after adjusting to sex. ALT and

AST were lower for men by 6.3% and 9% while in women by 3% and 5%, respectively. Generally compared with nonconsumers, high coffee consumers had statistically significant lower mean levels of liver enzymes and higher serum albumin (Gunter 2017). Ikeda et al. and Danielsson et al. (2013) observed that inverse association of coffee intake and liver enzymes activity is even more significant in people who consume alcohol compared with nonalcoholic drinkers (Ikeda et al. 2010; Danielsson et al. 2013). This phenomenon seems to have general application, as research around the world considering all human races, Caucasians, Black, White, Latinos, and others, confirm those findings. Setiawan et al. (2015, 2017) in recent multiethnic studies noticed that the reduction of liver enzyme activity and fibrosis is inversely associated with coffee consumption. This association was mainly observed in alcoholic liver disease when cirrhosis has already occurred, but in case of NAFLD, the association was significant for both cirrhosis and non-cirrhosis cases.

Due to recent EPIC studies, coffee drinking is strictly related with all-cause lower mortality in dose-dependent manner. In big cohort study lasting for 16.4 years, 520,000 participants from 10 European countries were investigated in case of mortality of any reason. Participants were divided into five groups depending on the coffee drinking amount: nonconsumers (NC) and drinking <1 (L), 1 – <2 (M-L), 2 – <3 (M-H), and 3+ (H) cups of coffee a day. Other factors were considered, like BMI, physical activity, smoking status and its intensity, education, menopausal status, ever use of oral contraceptives or menopausal hormone therapy, alcoholic drinks, total energy, and eating habits including red, processed meat, fruit, and vegetable intake. Strong inverse associations were observed between high coffee consumption and deaths of specific diseases. Mostly visible was mortality limitations due to deaths from diseases of gastrointestinal tract including liver disease, NASH, and alcoholic cirrhosis. Medium-low (M-L) coffee consumption reduced digestive diseases by about 30% in both men and women, while medium-high (M-H) coffee consumers reached 54% and 33% less for men and women, respectively. High (H) coffee consumers died of digestive diseases relatively 59% and 40% less frequently for men and women, respectively (Gunter 2017).

Coffee drinking health benefits have been repeatedly reported and analyzed in numerous studies including meta-analyses and reviews. Most of them are consistent proving that drinking of three to four cups of coffee a day significantly reduces morbidity and mortality of liver diseases including NAFLD, cirrhosis, and chronic liver diseases secondary to HBV and HCV like hepatocellular cancer. Regardless to the etiology of hepatocellular carcinoma, coffee drinking inversely reduces the morbidity which was documented in several studies (Setiawan et al. 2015; Gelatti et al. 2005; Larsson and Wolk 2007). All observations are convergent and report about 40% risk reduction for people who drink about three to four cups of coffee a day.

Moreover, coffee consumption appears to have an association with decreased risk of colorectal cancer. Coffee, as well as its individual constituents caffeine, caffeic acid, chlorogenic acid, and kahweol, has shown a potentially protective action in colorectal cancer – the third leading cause of cancer-related death in the USA. Epidemiological studies confirm strong sex dependency in colorectal morbidity

and coffee intake. In this case clear results are hard to obtain as reports are often contradictory and results are significant only in well-defined groups. In a recent report, Bułdak et al. compiled current research presenting analysis of coffee impact on colorectal cancer morbidity. Out of 21 epidemiological studies presented, more than half showed no significant association between coffee drinking and colorectal cancer incidence (Bułdak et al. 2018). Dose dependency was not observed even in Finland where coffee consumption reaches even ten cups a day (Bidel et al. 2010). The inverse association was reported in seven studies, while dose-dependent increased risk was observed in three studies. Sex dependency was reported in some studies, but information was inconclusive. Japanese studies of 96,162 people with 50,139 women report that risk of invasive colon cancer in woman who regularly drink three or more cups of coffee a day reach RR of 0.44 (95% CI = 0.19–1.04; p for trend = 0.04) compared with RR = 1.00 for non-coffee drinkers (12). In turn, in European research, a positive association was observed with HR = 1.12 (95% CI = 1.03–1.23) and HR = 1.33 (95% CI = 1.22–1.46) for three and four cups of coffee per day, respectively (Gunter 2017). It is difficult to confirm if ethnical differences are significant as inaccuracies occur even within homogeneous groups. Yamada et al. showed high risk of colon cancer in men, but not in women, drinking more than three cups of coffee a day with HR of 1.26 (95% CI 0.93–1.70), while Nakagawa-Senda et al. in the same dosage of three cups of coffee a day report of lower cancer risk of OR = 0.78 (95% CI: 0.65–0.92) (Yamada et al. 2014; Nakagawa-Senda et al. 2017).

The bioavailability and pharmacokinetic aspects of coffee may play an important role in colorectal cancer activity. In humans, one third of chlorogenic acid is absorbed in the small intestine, and the undigested rest is reaching the colon where it is hydrolyzed to caffeic acid by colon cancer cells. In turn caffeine is absorbed in the stomach and small intestine, and its oral bioavailability reaches 99% (Tian et al. 2019; Liguori et al. 1997). Caffeine present in serum is significantly inversely associated with colorectal cancer, but inverse association for decaffeinated coffee and colorectal cancer indicates that caffeine impact in vivo is lower as it is in in vitro studies. Kahweol in humans is absorbed in about 70% and plays a great role in carcinogenesis as protective agent against severe toxins like aflatoxin B1 or 2-amino-1-methyl-6-phenylimidazo(4,5-b)-pyridine. In fact, epidemiological studies focus on the entire coffee drink without precise qualification of individual constituents (Stalder et al. 1990; Lee and Jeong 2007).

Moreover, it is documented that chlorogenic acid may reduce intestinal glucose absorption and significantly affect glucose administration and its blood level. It becomes relevant as development of type 2 diabetes is strictly associated with liver diseases: in NAFLD and NASH patients it is estimated to be 22.51% and 43.63%, respectively (Younossi et al. 2016). It has been proved that coffee consumption is inversely associated with risk of type 2 diabetes regardless of caffeine presence. Drinking of six cups of coffee a day may lower the type 2 diabetes risk even on 33% with no statistical significance between caffeinated and decaffeinated one. This association may be even stronger, while high coffee drinking is usually connected with less healthy lifestyle. Ding et al. in his meta-analysis findings on

1,109,272 participants and 45,335 cases of diabetes had also noticed high dose dependency. For each cup of coffee per day, irrespective of caffeine content, the type 2 diabetes risk was lower at about 6–10%. This would indirectly imply health benefits of coffee distinguishing other compounds rather than caffeine (Ding et al. 2014).

22.8 Patents, Marketed Products, and Perspectives

Being well-known and important constituents of coffee and several vegetables, chlorogenic acid and related phytochemicals have been subjects of an immense number of patents around the world. A database search using "chlorogenic acid" yields over 12,000 hits, thereof 3500 are from the World Patents Office. Their scope is from isolation and purification from various materials (mainly coffee, though) to pharmaceutical and nutraceutical applications.

The pharmaceutical/therapeutic indications include polycystic ovary syndrome (WO2014025864A2), conjunctivitis (WO2009045054A2), metabolic syndrome (WO2008115723A1), fibroblast stimulation (WO2016150356A1), transglutaminase inhibition (WO2008153318A2), and many others. CGA was also patented by global corporations as ingredient of sweeteners that were supposed to prevent and combat inflammation, diabetes, and related disorders (e.g., WO2007061803A1, WO2008057968A2, WO2007061912A2).

Certainly, there is also a huge number of marketed products, claiming a lot of extraordinary health benefits. Most of the so-called "green coffee" supplements include chlorogenic acid activities in their advertisements. However, there are also products that are marketed as true herbal medicines, following the country-specific regulations. Chlorogenic acid or other compounds such as cynarin are quality markers in products from several herbal drugs, including pharmacopoeial ones. Such herbs include, for example, those listed in European Pharmacopoeia monographs: artichoke leaf, narrow-leaved coneflower root, nettle leaf, pale coneflower root, purple coneflower herb, and purple coneflower root. In Turkey, the bark from *Viburnum opulus* is used for manufacturing chlorogenic acid-enriched herbal drugs. Cardoon (artichoke) leaves are used in several European countries to prepare herbal medicines with indications for digestive and liver disorders.

This trend, however, does not imply that all these inventions and products indeed contribute to the importance of CGA and other CQAs as public health-relevant compounds. Nevertheless, it confirms the worldwide interest in these phytochemicals which will most likely continue in the future along with more insightful data on the mechanisms at work in the pharmacological and dietary properties attributed to consumption of coffee, Compositae vegetables, or, more recently, blueberries and bilberries. The latter have been not so intensively studied with respect to CQAs as important phytochemicals, contributing to their beneficial properties along with much more conspicuous anthocyanins.

As a closing remark, we suggest that instead of introducing too many preparations with unverified therapeutic or preventive properties, the future direction should

rather be to popularize CQA-rich foodstuffs, especially vegetables and herbal teas, in which CQAs could act in concert with other dietary polyphenols, therefore providing the consumers with broader scope of benefits. The concentrated extracts, with proper validation for CQA content, could also benefit the public health by preventing lifestyle diseases, improving longevity, and alleviating stress-related deterioration of health.

Whether this would be achieved by increased coffee consumption only is rather doubtful (depends on the local traditions and economic situation), and focus should be on change of dietary habits toward higher intake of selected vegetables and fruits such as blueberries and bilberries.

However, more epidemiological studies are needed population-wide to prove these beneficial effects of CQAs.

22.9 Cross-References

- ▶ Coumaric and Cinnamic Acids in Food
- ▶ Introduction of Phytonutrients
- ▶ Phenylpropanoids (Phenylpropenes) in Diets

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Coumaric and Cinnamic Acids in Food

23

Nataša Poklar Ulrih, Iztok Prislan, and Blaž Cigić

Contents

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Abstract

Cinnamic and coumaric acids belong to the large family of phenylpropanoids with C6-C3 skeleton. Main biosynthetic route involves deamination of phenylalanine into trans-cinnamic acid, followed by hydroxylation into coumaric acids. In plants, *p*-coumaric acid is a predominant isomer that is preferentially bound to cell wall polysaccharides by ester bond. Food processing in general results in the release of bound acids that can be absorbed in the gastrointestinal tract. The maximum plasma concentration of coumaric acid is reached in minutes after ingestion, and halftime in blood circulation is longer than that of more hydroxylated cinnamates. Pure compounds as also complex plant extracts with high cinnamic and coumaric acid content have beneficial effects in amelioration of diabetes, carcinogenesis, or uncontrolled inflammation. Additionally normal physiological functioning of lipid and bone metabolism and neuroprotective and eye-protective effects were confirmed. Most experimental results were obtained on model animals and human and animal cell cultures; there is nevertheless lack of human intervention studies with pure compounds. Low acute toxicity in mammals and pronounced toxic effect on certain pathogenic microorganism, together with antioxidant activity of the molecules, point to the potential application for food conservation. A large number of patents related to the production and application of cinnamic and coumaric acids in food and other sectors were issued in the recent years.

Keywords

Phenylpropanoids · Phenolic acids · Cinnamic acid · Hydroxycinnamic acids · Oxidative stress-related diseases

23.1 Introduction

We would like to start this chapter by quoting Hippocrates "Let food be the medicine and medicine be the food." Historically, natural products have played an important role in drug discovery to combat a variety of diseases. An oxidative stress, resulting from the imbalance between reactive oxygen and nitrogen species (ROS or RNS) and antioxidants, has been recognized as a major contributing factor in the pathogenesis of various diseases. Phenolic compounds are well-known antioxidants that scavenge and therefore potentially protect against ROS- or RNS-induced in vivo damage. Numerous studies have been performed to explore the positive role of polyphenols in the prevention and treatment of oxidative stress-related diseases, such as neurodegeneration, cardiovascular diseases, and cancer.

Phenolic acids are diverse group of secondary plant metabolites that are widely distributed through the plant kingdom and are an integral part of human diet. In food plants, they are occurring as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxy fatty acids, sterols, and

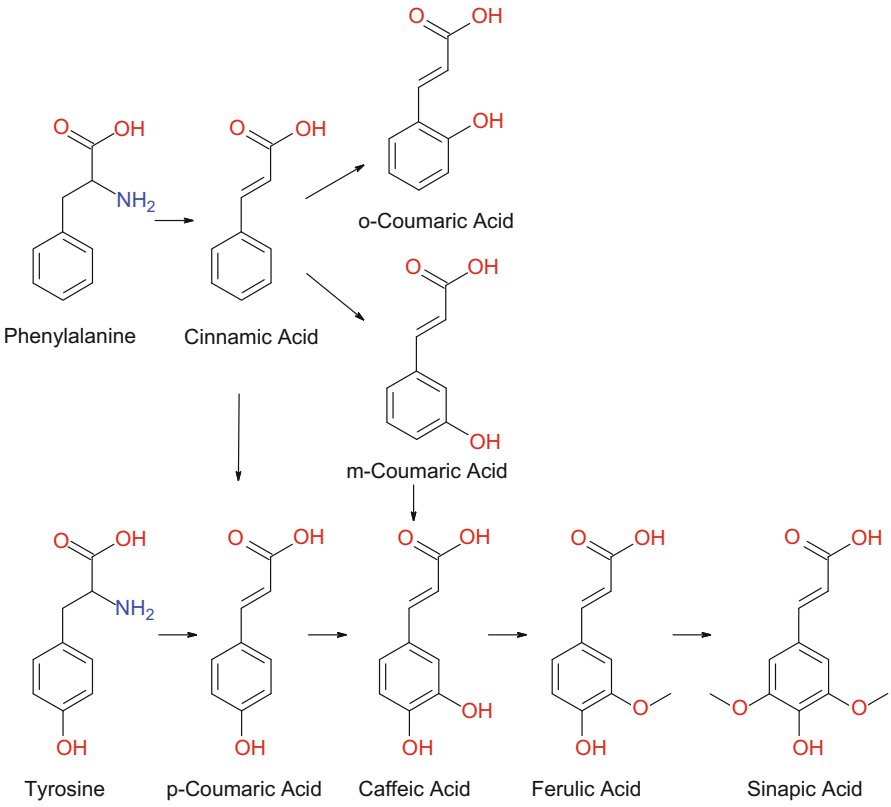

Fig. 1 Biosynthetic pathway of the phenylpropanoids in plants

glucosides. Although the role of phenolic acids in plants still remains unknown, they have been associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, structural components, and allelopathy (Naczk and Shahidi 2006). Phenolic acids can be divided into two major groups: hydroxybenzoic acids and hydroxycinnamic acids. Chemically, phenolic acids have at least one aromatic ring in which at least one hydrogen is substituted by hydroxyl group. The hydroxycinnamic acids are a series of trans-phenyl-3-propenoic acids that differ in their ring substitutions and include caffeic acid (3,4-hydroxycinnamic acid), ferulic acid (3-methoxy, 4-hydroxy), sinapic acid (3,5-dimethoxy, 4-hydroxy), and *p*-coumaric acid (4-hydroxy) (Fig. 1). There are three isomers of coumaric acid, *o*-coumaric acid (*o*-CouA), *m*-coumaric acid (*m*-CouA), and *p*-coumaric acid (*p*-CouA), that differ by the position of the hydroxy substitution of the phenyl group. *p*-CouA is the most abundant isomer of the three in nature and the most studied one. *p*-CouA exists in two forms, trans-*p*-CouA and cis-*p*-CouA. In different foods the derivatives of *p*-CouA such as *p*-coumaroyl glucose, *p*-coumaroyl malic acid, *p*-coumaroyl glycolic acid, *p*-coumaroyl tartaric acid, *p*-coumaroylquinic acid, and some others can be found (Fig. 2).

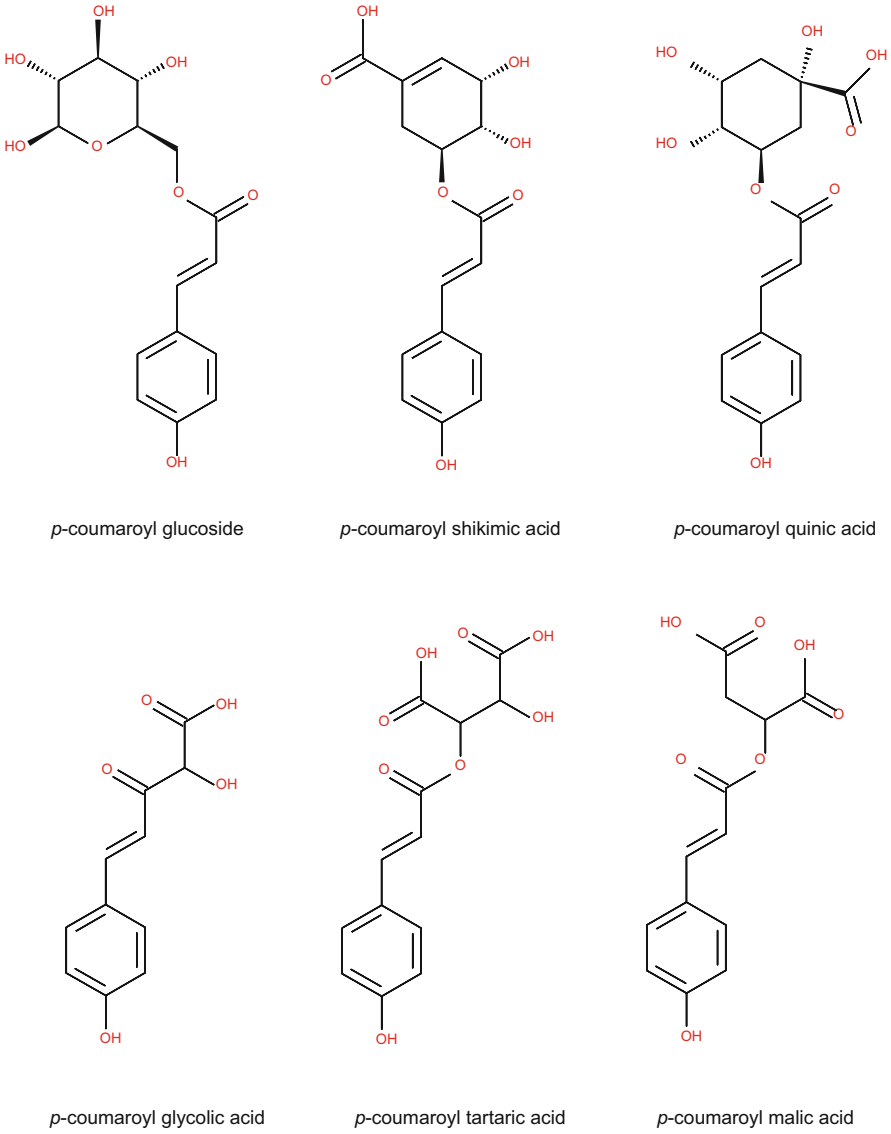

Fig. 2 Derivatives of *p*-coumaric acid

In Tables 1 and 2, the selected foods from Phenol-Explorer database which contain the highest amount of *p*-CouA and cinnamic acid (CinA) are listed, respectively. Detailed inspection of Tables 1 and 2 reveals that the content of CouA and CinA increased after hydrolysis process indicating that the *p*-CouA is conjugated (Neveu et al. 2010; Rothwell et al. 2012; Rothwell et al. 2013). Food processing as freezing, steaming, (pressure) boiling, frying, sterilization, and fermentation releases the cell wall-bound phenolic acids.

Table 1 Content of coumaric acid in foods. (The data were obtained in March 2019 from the http://phenol-explorer.eu/contents/polyphenol/454)

Food	Content of free *p*-coumaric acid	Content of *p*-coumaric acid after hydrolysis
Alcoholic beverages		
Wine (red)	0.55 mg/100 mL	3.02 mg/100 mL
Cereals and cereal products		
Rye, whole grain flour	0.23 mg/100 g FW	4.05 mg/100 g FW
Maize, refined flour	0.33 mg/100 g FW	3.51 mg/100 g FW
Rice, refined flour	0.11 mg/100 g FW	–
Fruits and fruit products		
Date, dried	5.77 mg/100 g FW	
Fruits – berries		
American cranberry	1.08 mg/100 g FW	25.38 mg/100 g FW
Seasonings		
Common sage, dried	4.95 mg/100 g FW	
Oregano, dried	5.75 mg/100 g FW	
Seeds/nuts		
Peanut, dehulled, roasted	6.46 mg/100 g FW	
Vegetables – fruit vegetables		
Olive (black), raw	*p*- 1.43 mg/100 g FW *m*- 12.50 mg/100 g FW *o*- 0.50 mg/100 g FW	13.00 mg/100 g FW – –
Olive (green), raw	*p*- 5.90 mg/100 g FW *m*- 8.00 mg/100 g FW *o*- 10.00 mg/100 g FW	10.50 mg/100 g FW – –

Table 2 Content of free *cinnamic acid* in foods. (The data were obtained in March 2019 from the http://phenol-explorer.eu/contents/polyphenol/454)

Food	Content of free *cinnamic* acid	Content of *cinnamic* acid after hydrolysis
Fruits – berries		
American cranberry	0.16 mg/100 g FW	2.05 mg/100 g FW
Strawberry, raw	0.22 mg/100 g FW	0.90 mg/100 g FW
Lingonberry, raw	4.12 mg/100 g FW	
Spices		
Chinese cinnamon	20.10 mg/100 g FW	–
Vegetables – fruit vegetables		
Olive (black), raw	0.77 mg/100 g FW	9.50 mg/100 g FW
Olive (green), raw	14.33 mg/100 g FW	21.00 mg/100 g FW

Presence of phenolics in foods may have important effects on the oxidative stability and microbiological safety of products. In addition, many phenolics in raw or processed foods have important biological activity related to their inhibitory

effects on carcinogenesis and other diseases. The *p*-CouA acid has beneficial effects on human health through the prevention of degenerative pathology, such as cardiovascular diseases and cancer (Kadoma and Fujisawa 2008). The beneficial effects of cCouA and CinA will be discussed in this chapter.

23.2 Bioactive Constituents

23.2.1 Biosynthetic Pathway

The phenylpropanoids are a diverse family of organic compounds that are synthesized by plants from the amino acids L-phenylalanine and L-tyrosine. Among the most widely distributed phenylpropanoids in plant tissues are the cinnamic acid (CinA) and hydroxycinnamic acids or hydroxycinnamates (HCAs) – a class of aromatic acids having a C6-C3 skeleton. The typical examples of HCAs are coumaric acid (hydroxycinnamic acid), caffeic acid (3,4-dihydroxy-cinnamic acid), ferulic acid (4-hydroxy-3-methoxy-cinnamic acid), and sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) which are produced from the shikimate pathway from L-phenylalanine or L-tyrosine as shown in Fig. 1. The most common HCAs are not present in plants in a free state but are predominantly associated with other molecules to form glycosylated derivatives or esters of tartaric acid, quinic acid, shikimic acid, glucose, or coenzyme A. Glycosylation takes place on carboxyl and not at the phenolic hydroxyl groups (Fig. 2).

L-Phenylalanine and L-tyrosine act as C6-C3 building blocks and are precursors for a wide range of natural products. In plants elimination of amino group from phenylalanine leads to formation of CinA, while elimination of amino group from tyrosine could yield *para*-coumaric or 4-coumaric (*p*-CouA) as shown in Fig. 1. All plants appear to have the ability to deaminate phenylalanine via phenylalanine ammonia lyase (PAL) enzyme and hydroxylate CinA into *o*-, *m*-, or *p*-CouA via cinnamic acid hydroxylase enzymes. On the other hand, the corresponding transformation of tyrosine yields only *p*-CouA and is mainly limited to members of the grass family (the Gramineae/Poaceae) (Pereira et al. 2009). *p*-CouA can be further oxidized via *p*-coumaric acid 3-hydroxylase (also called *p*-coumaroyl ester 3-hydroxylase) leading to formation of caffeic, ferulic, and sinapic acid. The most representative HCA is caffeic acid, which occurs in fruits, vegetables, and coffee, mainly as an ester with quinic acid (chlorogenic acid or 5-caffeoylquinic acid) (Croft 1998; Fingerman and Nagabhushanam 2006).

23.2.2 Mechanism of Antioxidant Activity

Oxygen is necessary for aerobic respiration to break down food molecules and to release energy. But aerobic respiration is also accompanied with generation of series of reactive oxygen species (ROS). Reduction of oxygen through addition of electrons (often during mitochondrial electron transport) leads to formation of a number

of ROS including superoxide anion radicals ($\bullet O_2^-$), hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), hydroxyl ion (OH^-), and nitric oxide (•NO). ROS are continuously produced and can have a role in cell signaling pathways (Hancock et al. 2001), but they can also cause damage to cells or crucial biomolecules, thus leading to disease conditions. It has been established that simple phenolic acids, such as benzoic or cinnamic acids, and their derivatives have antioxidant and radical scavenging properties (Kilic and Yesiloglu 2013). Antioxidant and radical scavenging potency of molecules depend on the electronic structure. For example, caffeic, sinapic, ferulic, and coumaric acids have an additional -CH=CH- group between phenyl and carboxyl group when compared to protocatechuic, syringic, vanillic, and p-hydroxybenzoate. This additional double bond makes caffeic, sinapic, ferulic, and coumaric acids more potent antioxidants and radical scavengers than their analogues without -CH=CH- group between phenyl and carboxyl group. It may be that the -CH=CH-COOH linked to the phenyl ring plays a role in stabilizing the radical by resonance (Cuvelier et al. 1992; Natella et al. 1999; Mathew et al. 2015). According to the literature, the quasi-planar geometry of the molecule contributes to a better stabilizing effect through delocalization of the π-electron between aromatic ring and C=C bond (VanBesien and Marques 2003).

CinA and HCAs share a lot of properties and characteristics with polyphenols. Similar to polyphenols, CinA and HCAs are able to act as antioxidants and/or chemoprotectants. The mechanism of antioxidant activity can follow one (or combination) of several pathways shown in Fig. 3.

In the case of free radical scavenging, CouA can break free radical chain reaction. Hydroxyl group on benzene ring can react with reactive oxygen and nitrogen species by donating hydrogen and forming a radical form of CouA with unpaired valence

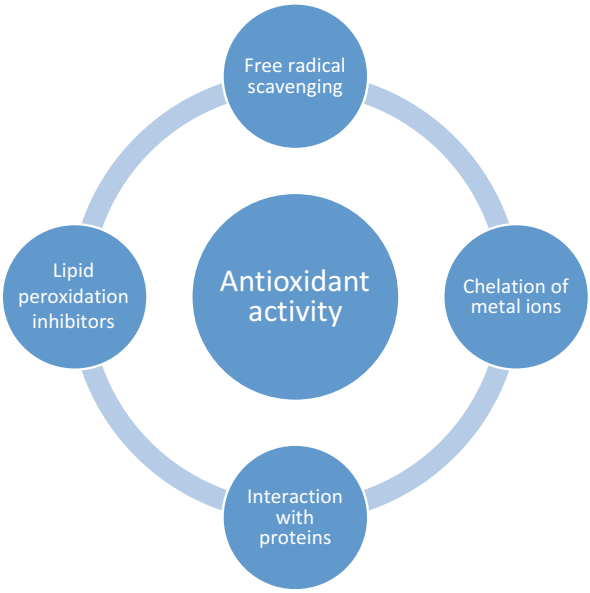

Fig. 3 Different mechanisms of antioxidant activity

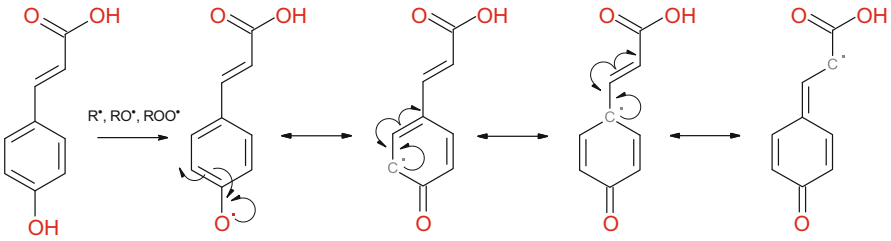

Fig. 4 Proposed mechanism of *p*-coumaric acid antioxidant activity

electron on oxygen atom. Radical form of CouA is more stable than reactive oxygen and nitrogen species because of the interaction of the hydroxyl group with the π-electrons of the benzene ring. Namely, radical form of CouA is stabilized by delocalization and resonance (Fig. 4). The formation of these relatively long-lived radicals is able to modify radical-mediated oxidation processes (Pereira et al. 2009; El-gizawy and Hussein 2017). The antioxidant activity of phenolic acids is related to the number and position of hydroxyl groups in the molecule. Even though CinA lacks any hydroxyl groups on benzene ring, it still possesses some radical scavenging activity. The ability to scavenge free radicals increases by adding hydroxyl group to benzene ring, thus obtaining CouA. Radical scavenging activity of CouA can be further optimized by placing hydroxyl group on *para*-position and/or adding second hydroxyl or methoxy group, thus obtaining other HCAs. Regardless of the position of hydroxyl group, CouA has lower antioxidant activity when compared to other HCAs (Briante et al. 2003) and can even behave like prooxidants, depending on their concentration (Fukumoto and Mazza 2000; Kristinová et al. 2009). The antioxidant efficiency of monohydroxylated compounds is strongly enhanced by the introduction of a second hydroxyl group at the ortho (e.g., caffeic acid)- or para-positions and is increased by one or two methoxy substitutions in ortho-position (e.g., ferulic or sinapic acid) with respect to the hydroxyl group (Fukumoto and Mazza 2000).

Exposure to environmental chemical carcinogens may be responsible for large number of human cancers. Nitrites from food and water are precursors in the formation of N-nitroso compounds, which are genotoxic compounds consisting of nitrosamines and nitrosamide. Formation of N-nitroso compounds can be blocked by all HCAs, including *p*-CouA. *p*-CouA can react with nitrites and thus function as chemoprotective agents to inhibit N-nitrosamine formation. Figure 5 shows the beginning of the mechanism of reaction of *p*-CouA with nitrite in H_2O that can lead to different products depending on the conditions. *p*-CouA is most reactive at acidic pH, but it might also serve as a nitrite scavenger at higher pH values (Torres y Torres and Rosazza 2001).

Due to absent hydroxyl group(s) on phenyl ring, the ability of CinA to scavenge free radicals is lower when compared to HCAs. Nevertheless, it can boost antioxidant response by elevating antioxidant enzyme levels. Consequently, the pretreatment with CinA can provide protection to normal cells from the toxic effects of the widely used anti-cancer drug by significantly reducing the lipid peroxidation.

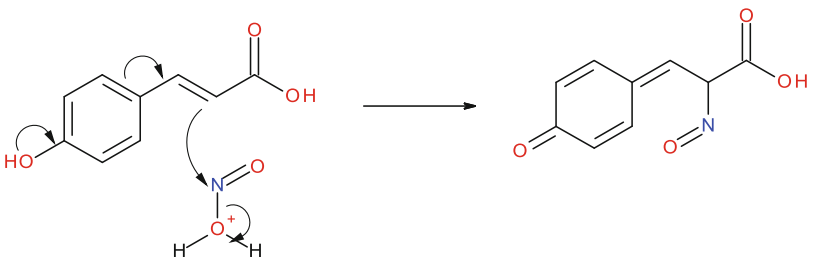

Fig. 5 Reaction of p-coumaric acid with nitrite in water which can lead to different products depending on conditions likely to be found in living systems. (Adapted from Torres y Torres and Rosazza 2001)

The details of protection mechanism offered by CinA are still under investigation (Patra et al. 2012). Although antioxidant activity of HCAs is most often linked to their hydrogen donating ability, other mechanisms have been suggested. The antioxidant capacity of HCAs is also attributed to their ability to chelate transition metals, like copper or iron, which act as catalysts in the production of free radicals leading to lipid peroxidation, protein modification, and DNA damage (Vaya and Aviram 2001; Andjelkovic et al. 2006; Gaspar et al. 2009). Catechol group seems to be crucial for complex formation, so CinA and CouA should not be unable to chelate metals. This seems to be true for CinA, but there are reports describing the ability of *p*-CouA to form complexes with several metals (Swislocka et al. 2012; Shen et al. 2018).

CinA and HCAs exhibit amphoteric properties due to their hydrophobic phenyl ring, and hydrophilic hydroxyl groups thus have the potential to interact with proteins. Interaction with proteins is important because it can modulate (a) nutrient uptake by solute-binding protein transportation (Tan et al. 2013), (b) quality of a food product during production and subsequent storage (Kaur et al. 2018), and (c) the activity of some specific enzymes, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenases, and xanthine oxidases (Cos et al. 1998; Pereira et al. 2009). CinA and CouA can reversibly bind to serum albumins using them as carriers, thus increasing their uptake and overcoming low aqueous solubility.

23.3 Bioavailability and Metabolism

Most bioavailability studies concern caffeic acid and ferulic acid although there are other dietary important HCAs like rosmarinic acid or caftaric acid. Cereals, coffee, fruits, and vegetables are main sources for dietary CinA and HCAs including *p*-CouA; ferulic, caffeic, and sinapic acid; and their esterified/etherified conjugates. Table 2 shows that the highest amounts of CinA can be found in cinnamon and green olives. High amounts of CouA are present in eggplant, olives, broccoli, and asparagus; other sources are sweet cherries, plums, blueberries, cranberries, citrus peel and seeds, and orange juice. Daily intake of total HCAs can be as high as 2000 mg

(Clifford 2004), but this number varies depending on country, personal dietary habits, and geographic location (Bravo 1998). Due to their high consumption, cereals, coffee, and tea seem to be the most important sources of HCAs. Kern et al. showed that consumption of a 40 g portion of commercial high-bran breakfast cereal provides ~120 mg of hydroxycinnamates (Kern et al. 2003), and Clifford estimated that a single cup of coffee may contain 70–350 mg of hydroxycinnamates (Clifford 1999). Main food contributors to HCA intake are mostly represented by coffee since a coffee drinker usually drinks more than one cup of coffee per day and consequently ingests on average 630 mg of hydroxycinnamates per day just from coffee (Grosso et al. 2014). In Western countries, coffee and products derived from this drink are consumed regularly, thus representing the main food source of HCAs.

HCAs are usually covalently bound to the cell wall by forming ester bond with polysaccharides or can be found in plants as esters of tartaric acid, quinic acid, shikimic acid, glucose, or coenzyme A. The free form is also present in plants, but its amount is always lower than the amount of esterified form. For example, the amounts of free HCAs in coffee pulp were as follows (in%): *p*-coumaric acid 2.8, caffeic acid 5.6, chlorogenic acid 23.1, and ferulic acid 26.6 with respect to total content of individual HCA(Teresa Torres-Mancera et al. 2011). Feruloyl esterases represent a diverse group of carboxyl esterases that catalyze the hydrolysis of ester bonds between HCAs and plant cell wall polysaccharide. These enzymes have been mostly studied from microbial sources as potential biocatalysts in a wide variety of applications such as in biofuel, food and feed, pulp and paper, cosmetics, and pharmaceutical industries, but they are also present naturally in plants (Dilokpimol et al. 2016). There are no esterases in human tissues able to break these ester links, but Couteau et al. showed that certain gut bacteria, including some already recognized as potentially health-promoting (i.e., species belonging to the genera *Bifidobacterium* and *Lactobacillus*), have feruloyl esterase activity and are involved in the release of free HCAs in the human colon (Couteau et al. 2001). Because esterified HCAs exhibit lower absorption and bioavailability than free HCAs, the esterase activity of gut bacteria is crucial for transport of HCAs into human bloodstream. Although the main site for metabolism of esterified HCAs is colonic microbiota, up to one third of their absorption can also take place in the small intestine (Borges et al. 2013; Marin et al. 2015). Unabsorbed esterified HCAs that reach the colon are metabolized by the colonic microbiota into free HCAs (Deprez et al. 2000; Ou and Gu 2014; Neilson et al. 2017). Over the length of the colon, esterified HCAs are slowly metabolized, and gradient of native compounds and their microbial metabolites is likely established (Tsang et al. 2005).

To explain the in vivo effects of these compounds, it is very important to know the fate of phenolic compounds in the human body. The bioavailabilities of phenolic compounds vary widely, which depend on their chemical structures, metabolism, and biological activities (Ota et al. 2011). In situ or ex vivo absorption models suggest that, unlike covalently bound, free HCAs can be absorbed in all sections of the rat gastrointestinal tract, including the stomach, jejunum, ileum, and colon (Zhao and Moghadasian 2010). The exact mechanism for absorption of CinA and HCAs is not entirely clear due to the complexity of the processes affecting the absorptive

processes including diffusion and gut transporters. Since monocarboxylic acid transporters (MCTs) are expressed in many tissues and have an important role in the intestinal absorption, they might be involved in the absorption of CinA, HCAs, and their derivatives. Intestinal absorption of various compounds is usually studied with the human colorectal adenocarcinoma cell line (Caco-2), which represent a useful model since the cells are morphologically and functionally similar to human small intestinal epithelial cells. Uptake of CinA and some of the HCAs has been studied, and it was discovered that the uptake of di-hydroxycinnamic acid was markedly lower than that of mono-hydroxycinnamic acids and that the uptake of mono-hydroxycinnamic acids was lower than that of CinA. In general, hydroxylation, particularly di-hydroxylation, decreased the affinity for MCTs, probably due to decreased lipophilicity (Tsukagoshi et al. 2018). There are evidences suggesting that the uptake of HCAs might involve passive diffusion (Poquet et al. 2008; Zhao and Moghadasian 2010). If this is the case, then bioavailability of free HCAs should be correlated with their polarity because large polar substances are unable to cross lipid bilayers. The results published by Ota et al. (2011) and Nardini et al. (2009) on bioavailability of the free HCAs show that *p*-CouA which is less polar than caffeic and ferulic acids has the highest effect on the structure of the membrane lipids. Caffeic and ferulic acids, both more polar than *p*-CouA, had smaller effects on rigidity and dynamics of phospholipid chains in the cell membrane structure.

In order to determine bioavailability of CinA and HCAs, their concentration is measured in plasma and urine after ingestion. Analysis of *p*-CouA from blood and urine confirmed that *p*-CouA was absorbed well in the gut and excreted in its original form. Bioavailability of *p*-CouA was estimated to be between 70% and 75%, which is relatively high (Konishi et al. 2004; Zhang et al. 2007). It takes only couple of minutes to reach maximum plasma concentration of *p*-CouA after oral administration. It takes a little longer for ferulic acid to reach maximum plasma concentration meaning that *p*-CouA is absorbed more rapidly than ferulic acid. *p*-CouA is absorbed throughout the digestive tract with passive transport being the major pathway by which *p*-CouA enters bloodstream. On the other hand, an extensive absorption of CinA by the duodenum, jejunum, ileum, colon, and cecum has been associated with active transport system and indication that MCT may play a part in CinA absorption. It seems that absorption of CouA is slower and less efficient than that of CinA (Garrait et al. 2006). The metabolism of CinA and HCAs may occur in the liver, intestinal mucosa, and kidney and/or by intestinal microflora. HCA may undergo several enzymatic reactions during its metabolism, including O-methylation, sulfation, glucuronization, GSH conjugation, glycination, dehydroxylation, demethylation, dehydrogenation, and hydrogenation. After the free acid is released by bacterial esterases, it is then metabolized to reduce the double bond. The reduction of double bond yields phenylpropionic acid, which is then decarboxylated to produce phenylacetic acids. In the next step, dehydroxylation removes the hydroxyl at the 4th C-atom of the HCA phenyl ring. Methyl groups can also be removed from phenyl ring through demethylation at different stages of degradation (Tomás-Barberán et al. 2009). However, majority of *p*-CouA stays in free form after absorption and doesn't seem to become glucuronated by the jejunum

cells (Spencer et al. 1999). Ten minutes after oral administration of *p*-CouA, 58% of free form is still present in plasma. This percentage drops to 50% within 60 min of oral administration (Konishi et al. 2004). By contrast, free ferulic acid decreases to 15% within 15 min and to 1% within 60 min after oral administration (Zhao et al. 2003); thus the conjugation of *p*-CouA appears to be slower than that of ferulic acid. This can be confirmed by comparing urinary excretion rate of free *p*-CouA (24%) and free ferulic acid (5.4%) in rat urine. Plasma metabolites of ferulic acid were mainly sulfates or sulfoglucuronides regardless of the form under which ferulic acid was administered (free or combined with different carbohydrates). Absorbed free *p*-CouA can also be conjugated with glucuronide, sulfate, and sulfoglucuronide in the liver (Pei et al. 2016).

23.4 Bioactivities

23.4.1 General

Bioactivity of CinA and HCAs related to health has been subject of many reviews in the recent years. This is not surprising as research within this field is extremely active. Recently there was a shift from a study of general antioxidant properties to a specific influence on the different pathological processes, mostly on chronic and systemic disorders. In the case of coumaric acid, a relative proportion of word "coumaric acid" as also health-related words in the abstracts of manuscripts published in journals with impact factor is higher in last few years (Table 3). Experiments performed on human and animal cell cultures and dietary interventions with animals have shown many health-related benefits. Addition of CouA resulted in slower growth and spreading of tumors, often related to induced apoptosis of cancer cell and antiangiogenic activity. CouA has also antidiabetic and antihyperlipidemic activity and is therefore a potentially useful dietary component to ameliorate the negative effects of type 2 diabetes and obesity. Studies on various cell lines and dietary interventions on model animals have shown that coumaric acid possesses immunomodulatory and anti-inflammatory properties. The neuroprotective effects

Table 3 Comparison of particular word usage related to coumaric acid and health-related properties in the abstracts of manuscripts published in journals with impact factor. The data were obtained in February 2019 from the Web of Science database for indicated time periods

	All years	2014–2018	2016–2018
All manuscripts	33,723,429	10,190,679	6,301,026
Coumaric	5708	2084	1360
Cancer	294	161	109
Inflammation	98	68	49
Apoptosis	119	72	55
Diabetes	61	38	32
Health	402	234	163

mostly related to amelioration of ischemia were also confirmed. Those and some other health beneficial properties are presented in this section. The bioactivity-related properties of CinA were less analyzed; nevertheless some beneficial effects were also observed.

23.4.2 Cancer

23.4.2.1 Animal Cell Cultures and Model Animals

Certain phenolic compounds have anticancer properties. They can influence various stages of cancer development: transformation of normal cell into cancer cells, growth of tumors, and spreading of tumors from primary site to other sites within the organism (Anantharaju et al. 2016).

p-CouA can form hydrogen bonds to a heterocyclic aromatic amine that is formed during thermal treatment of foods and reduce its binding to DNA that could result in frameshift mutations (Ferguson et al. 2003). At 150 μmol/L *p*-CouA induce apoptosis of mouse neuroblastoma cancer cells lines. Large enhancement of ROS production resulted in structural changes of mitochondrial membrane and induction of caspase-8 mRNA (Shailasree et al. 2015). Angiogenesis is a crucial factor for tumor growth and migration. Methyl ester of *p*-CouA showed a dose-dependent anti-angiogenic activity on human umbilical vein endothelial cells inhibiting proliferation and tube formation in submillimolar range. Same compounds were tested on zebra fish embryos where a potent inhibition of vessel formation and inhibition of angiogenesis in zebra fish tumor cell line were observed already at 5–20 μmol/L range (Zhang et al. 2018). Mechanism of anticancer activity of *p*-CouA could be related also to the inhibition of indoleamine 2,3-dioxygenase expression that is overexpressed in various types of human cancers.

Animal feeding studies with *p*-CouA included in diet show mixed results. Rats fed with high-fat diet and incorporated *p*-CouA (0.1% w/w) developed less colorectal carcinomas and more adenomas induced by dimethylhydrazine in comparison to control; however, none of the effects was statistically significant (Femia et al. 2005). Later it was shown that *p*-CouA at a daily dose in a range 50–200 mg/kg of body weight statistically significantly inhibit formation of preneoplastic lesions induced by dimethylhydrazine. The onset of carcinogenesis is most likely attenuated by dose-dependent induction of antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase (Sharma et al. 2017). Stabilization of redox status in normal cells that are affected by anticancer therapy is one of the benefits of *p*-CouA. Cisplatin is the potent anticancer agent that increases oxidative stress. *p*-CouA fed to adult Wistar rats was shown attenuate nephrotoxicity and hepatotoxicity, by increasing the expression of antioxidant enzymes that were down regulated by cisplatin (Akdemir et al. 2017). Such action of *p*-CouA can nevertheless be a double-edged sword, as improved redox status help to survive not only normal cells but also cancers cells and could therefore in principle promote carcinogenesis.

Cinnamic acid intragastrically administered to mice (150 mg/kg; 6 doses in 2 weeks) efficiently reduced the growth of colon carcinoma xenografts without having

the influence on body weight. The antitumor effect was attributed to the inhibition of histone deacetylases (Zhu et al. 2016).

23.4.2.2 Human Cell Cultures

Redox imbalance is one of the key mechanisms in cancerogenesis. High concentration of ROS can lead to mutagenesis, but also trigger necrotic or apoptotic death of cancer cells. On the other hand, lower concentration of ROS inhibits cell migration and proliferation but also increases the survival rate of cancer cells. In this regard anticancer potential of molecules that can alter the redox status of cells should be taken with care (Helfinger and Schroder 2018).

Experiments on human cell cultures have shown that *p*-CouA can inhibit growth of colon cancer and lung cancer cell lines in micromolar and millimolar range. Interestingly at millimolar range, the inhibition of growth was attributed to the induction of apoptosis through overproduction of ROS in mitochondria (Jaganathan et al. 2013), whereas, in hundred micromolar range, lower cell viability was attributed to the decreased concentration of superoxide anion that is crucial for normal functioning of cancer cells (Bouzaiene et al. 2015). Addition of *p*-CouA to the cell culture growth media resulted in lower antiangiogenic potential of endothelial cells (Kong et al. 2013). The viability of two different treated human cancer cell lines was significantly reduced from 10 micromolar to millimolar concentration range of *p*-CouA in growth media. Under these conditions, *p*-CouA downregulated the expression of molecular chaperone Grp78, concentration of which is increased in cancer cells. Lower concentration of Grp78 led to apoptosis (Sharma et al. 2018). Findings on cell cultures were confirmed on model animals. Male albino Wistar rats fed on a daily dose of *p*-CouA (100 mg/kg) showed significantly lower number of colon tumors induced by dimethylhydrazine and similarly to cell cultures lowered expression of Grp78. Anticarcinogenic effect on human breast cells was shown also for *o*-CouA that is positional isomer of *p*-CouA; nevertheless the IC_{50} value was relatively high (5 mmol/L) (Sen et al. 2013). Glucoside of *p*-CouA that is found in Chinese traditional herb decrease the viability of various human cancer cell lines with IC_{50} values in hundred micromolar range. The effect was attributed to mitochondria-mediated apoptosis. Same compound fed to mice at 50 mg/kg resulted in substantial reduction of tumor size (Peng et al. 2015). Alky esters of *p*-CouA that can be found in the roots of some edible plants as sweet potato are extremely cytotoxic to certain human cancer lines. The IC_{50} values for lymphoblastic leukemia cells are in sub micromolar range, even an order of magnitude lower than cisplatin, a chemotherapeutic agent. They induce apoptosis and activate caspase-3 enzyme (Menezes et al. 2017).

Cinnamic acid decreases the viability of human cancer stem cells that are often resistant to drug treatment. Incubation of human colorectal cancer cells in the presence of cinnamic acid at concentration higher than 10 mM resulted in reduced cell viability (IC50 13.6 mM). Nevertheless such concentrations cannot be obtained in vivo and are also for two to three orders of magnitudes higher than those of some common chemotherapy drugs (Soltanian et al. 2018).

23.4.2.3 Results Obtained on Complex Mixtures of Polyphenols with High p-CouA Content

Anticancer potential was also shown for various complex mixtures of phenolic compounds in which *p*-CouA is one of the major compounds. Cell wall-bound polyphenols of foxtail millet increased the sensitivity of human colorectal cancer cells to various chemotherapeutic agents. The mechanism is attributed to the lower expression of some drug resistance proteins, inhibition of cell proliferation, and promotion of apoptosis (Lu et al. 2018). Onion peel extract (100 mg/L) with high content of *p*-CouA had a strong cytotoxic effect on human colon carcinoma cells. Additionally onion peel extract lowered the synthesis of some antioxidant enzymes that were induced by exogenous oxidative stress and have an anti-inflammatory activity (Kim et al. 2013b). The extract of the herb *Salicornia freitagii* has a cytotoxic effect on human colon carcinoma cells. The mechanism is attributed to modulatory effect on the expression of enzymes involved in detoxification pathways and xenobiotic activation (Altay et al. 2017). Propolis, a complex mixture of phenolic compounds, where *p*-CouA is a mayor phenolic acid constituent, showed an apoptotic effect on human tongue squamous cell carcinoma cells. The ethanolic extract of propolis activated caspases 3, 8, and 9 in a dose-dependent manner. Mixture of constituents phenolic compounds was more potent inducer of apoptosis in comparison to particular compound indicating a synergistic effect (Czyzewska et al. 2016). Further experiments revealed that induction of apoptosis by phenolic compounds in propolis is related to increased mitochondrial proline degradation and decreased proline availability for collagen biosynthesis (Celinska-Janowicz et al. 2018).

Cinnamomum cassia bark extract, where cinnamic acid is a major constituent, induced apoptosis in human and mice oral cancer cells already at concentration of 25 mg/L. The extract supplied to mice by oral gavage (250 mg/day) efficiently reduced the tumor growth in mice, without having influence on weight (Yu et al. 2019).

23.4.3 Diabetes

23.4.3.1 Animal Cell Cultures and Model Animals

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism. *p*-CouA has been shown to ameliorate some of the negative impacts of high glucose level and also interferes with some signaling pathways that are involved in the onset of type 2 diabetes. At 25 μM *p*-CouA improved glucose uptake by adipocytes in combination with hypoglycemic drug and increased expression of glucose transporter GLUT4 by threefold. Additionally expression of fatty acid synthase and HMG-CoA reductase, key enzymes in fatty acid, and cholesterol biosynthesis was reduced (Prabhakar and Doble 2011).

Incorporation of *p*-CouA into diet of rats resulted in the amelioration of certain complications of diabetes. Diabetic rats consuming 40 mg/kg of *p*-CouA for 6 weeks have statistically significant lower glucose and glycosylated hemoglobin and higher

insulin levels. Intake of *p*-CouA also resulted in decreased level of proinflammatory signal molecule TNF-α (Abdel-Moneim et al. 2018). Same treatment of diabetic rats also resulted in lower brain oxidative stress and declined inflammation and apoptosis in hippocampus region that is important for short- and long-term memory and is one of the first regions of the brain that is damaged in dementia (Abdel-Moneim et al. 2017). Daily administration of *p*-CouA (100 mg/kg) to rats with the experimentally induced diabetes led to reduced levels of blood glucose and higher insulin and also higher activity of glycolytic enzymes and lower activity of gluconeogenic enzymes in the liver. Statistically significant decrease in cholesterol and triglycerides in the kidney and liver was also observed (Amalan et al. 2016). Incorporation of mulberry leaves (5%) that are used for culinary purposes and brewing of tea into the diet of diabetic rats resulted in significant decrease in blood glucose levels and improved kidney function. Feeding on mulberry leaves resulted in reduction of the PPAR-gamma levels in kidneys and increase in phosphorylation of regulatory important Ser112, the receptor that regulates fatty acid and glucose metabolism. Incorporation of mulberry leaf extract (20 μM gallic acid equivalent) and its main phenolic constituent p-CouA (5 μM) into growth media resulted in the increased phosphorylation of Ser112 in PPAR-gamma expressed in the canine kidney cell line. Such experiment undoubtedly shows that *p*-CouA can interfere with inflammation and diabetes (Gurukar and Chilkunda 2018).

CinA have even more pronounced antidiabetic properties than *p*-CouA. Diabetic rats fed orally with CinA (10 mg/kg) 1 h prior to glucose intake (3 g/kg) had much lower increase in blood glucose in comparison to control. The magnitude of the effect 2 h after glucose intake was of the same range as 5 mg/kg intake of antidiabetic drug glibenclamide (Hafizur et al. 2015). As intake of cinnamaldehyde that is aside from CinA the major constituent of cinnamon bark extracts did not result in lower blood glucose, the antidiabetic properties of cinnamon could be related to cinnamic acid. Beneficial effects of CinA in type 2 diabetes are not related only to regulation of blood glucose level. CinA is also an efficient antiglycation agent. At 1 mmol/L concentration, it significantly inhibited formation of advanced glycation end products in vitro. The magnitude of the effect was much more pronounced in comparison to all three positional isomers of CouA (Adisakwattana et al. 2012).

23.4.4 Lipid Metabolism

23.4.4.1 Animal Cell Cultures and Model Animals

p-CouA and some complex plant extracts with high content of *p*-CouA interfere with lipid metabolism and can ameliorate some negative impacts of obesity and hyperlipidemia. *p*-CouA has a large impact on metabolism of rat skeletal muscle cell lines above 10 μM concentration. It promotes beta-oxidation of fatty acid, suppressed triglyceride accumulation, and enhanced glucose uptake (Yoon et al. 2013). Incubation of mouse preadipose and muscle cell lines at submillimolar concentration of *p*-CouA suppressed differentiation of these cells by downregulating

many transcriptional factors. It was also cytotoxic to preadipocytes above 500 μM concentration. Specifically the expression of PPAR-gama2 was decreased, and it was shown by molecular modeling that *p*-CouA could hydrogen bond to PPAR-gama2 (Ilavenil et al. 2016).

p-CouA at (18 mg/kg) added to diet of male rats fed on high-fat and high-fructose diet resulted in lower total blood cholesterol and triglycerides and better HDL/LDL ratio. Hepatic triglyceride was also reduced in comparison to control (Guo et al. 2017). Supplementation of *p*-CouA (100 mg/kg) to high-fat diet in rats showed reduction on body mass and adipose tissue in particular. Markers of oxidative stress and blood lipid composition were also significantly improved (Hsu et al. 2009). Dietary *p*-CouA (0.2 w/w) lowered plasma and liver lipid levels and promoted the fecal excretion of sterols (Yeh et al. 2009).

Not only pure *p*-CouA but also some complex plant extracts with high content of *p*-CouA can regulate lipid metabolism. Daily administration of pineapple leaf extract (200–800 mg/kg) rich in polyphenols and particularly *p*-CouA (1.5%) significantly reduced accumulation of abdominal fat and accumulation of lipids in the liver. Expression of carnitine acyltransferase 1 (CAT1: catalyze essential step in beta-oxidation) and also some other enzymes is included in mitochondrial catabolism. Experiments on human liver cell cultures revealed that also pure *p*-CouA has similar effect on induction of CAT-1 and inhibited intracellular accumulation of fat (Xie et al. 2014). Similar results were obtained for aqueous extract of hulled barley. When injected intraperitoneally (15–50 mg/kg) barley extract in dose depended manner inhibited differentiation of adipocytes, fat mass and body weight gain in mice. Oral intake for 8 weeks (15 mg/kg) also resulted in lower weight gain. Anti-adipogenic effect of pure *p*-CouA (already at 1 μM) was confirmed on cell murine preadipocytes (Seo et al. 2015). *p*-CouA (28 mg/g) is the major phenolic compound in the extracts of kenaf (important fiber crop) seed meal. Incorporation of the extract (2.3–4.6%) or meal itself into high-fat diet of rats resulted in improvement of hepatic and kidney function, lower accumulation of liver fat, total and LDL serum cholesterol, and higher HDL cholesterol (Chan et al. 2018). Dwarf bamboo extract (100–1000 mg/L) and its major constituent *p*-CouA (4 μM) attenuated accumulation of lipids in human-cultured liver cells by induction of phosphorylation of AMP-activated protein kinase and expression of CAT1, resulting in activation of catabolic pathways of lipid metabolism and inhibition of lipogenesis (Kim et al. 2013a). Welsh onion extract (50–400 mg/L) and its major phenolic constituent *p*-CouA (20–40 μM) interfered with cholesterol metabolism in human hepatic cell cultures. Both pure compound and extract contributed to the prevention of LDL receptor degradation at low concentration of lipoproteins. Therefore they are potentially effective in reducing hypercholesterolemia (Choi et al. 2017).

Trans-CinA at hundred micromolar range in growth medium has a significant influence on the preadipocyte differentiation. It induced brown-like phenotype in white adipocytes and activated brown adipocytes, resulting in increased beta-oxidation and thermogenesis and reduced lipogenesis. Therefore trans-CinA could be potentially useful in treatments of obesity (Kang et al. 2019).

23.4.5 Inflammation

Inflammation is a normal protective response to a certain stimulus as pathogens or damaged tissue. The functions of inflammation are to eliminate the invading microbes and necrotic cell and to initiate process of tissue repair which are all beneficial to the survival of organism. There is however a darker side of inflammation where it became a chronic process that plays an important role in many systemic disease as rheumatoid arthritis, atherosclerosis, cancer, diabetes, and others. *p*-CouA have an immunomodulatory and often an anti-inflammatory properties and could ameliorate the outcome of some chronic diseases.

23.4.5.1 Animal Cell Cultures and Model Animals

Proliferation of mice splenocytes (suspension of different white blood cells) was decreased by *p*-CouA in hundred-micromolar range (Kilani-Jaziri et al. 2017). Extracts of edible fern, an important wild vegetable in China with high content of *p*-CouA, decreased expression of interleukins, the important mediators of inflammatory response in mice macrophages (Dion et al. 2015).

Induction of intestinal inflammation in rats was suppressed when *p*-CouA was administered in daily dose of 50 mg/kg. The effect was attributed to the reduced expression of proinflammatory cyclooxygenase COX-2 (Luceri et al. 2004). *p*-CouA fed to rats 100 mg/kg per day showed an immunosuppressive properties and lowered expression of inflammatory mediator TNF-α in arthritic rats (Pragasam et al. 2013). Medicinal herb *Bambusae Caulis* extracts and its major phenolic constituent *p*-CouA, supplied by oral injection, inhibit cigarette smoke-induced inflammation in mice. Downregulation of the expression of proinflammatory molecules was observed (Kim et al. 2018). Atherosclerosis that can lead to severe cardiovascular complications was attenuated in rats on a high fat and high-sucrose diet, when daily dose of *p*-CouA (75 mg/kg) was supplied. Improvement in HDL/LDL ratio and hypoglycemic effect was found, and lower degree of histopathological alternations of aorta wall was observed (Omar et al. 2018). Excessive accumulation of urate in joints can lead to inflammation and arthritis. Daily consumption of *p*-CouA (100 mg/kg) by rats with urate-induced inflammation resulted in improved biochemical markers, lower inflammation, and better histopathology of ankle joint (Pragasam and Rasool 2013). Natural herb *Oleandia diffusa* and its major phenolic compounds *p*-CouA (14 mg/kg) fed to rats with induced arthritis have anti-inflammatory effects. Serum levels of inflammation mediators TNF-α and IL-6 decreased as also swelling, joint redness, and deformability (Zhu et al. 2018). Rats with induced rheumatoid arthritis benefited from the consumption of *p*-CouA (100 mg/kg). *p*-CouA significantly suppress joint edema and expression of inflammatory mediators. Enhanced bone mineral density and suppression of bone destruction and cartilage degradation were confirmed by radiological scans.

23.4.5.2 Human Cell Cultures

On the contrary to some experiment with mouse white blood cells where *p*-CouA showed inhibition of proliferation, viability of human monocytes was not affected

by *p*-CouA or propolis. The reason for such observations may be related to the two orders of magnitude lower concentration (0.5 μM) in study with human leukocytes. Even at such low concentration, statistically significant induction of some inflammatory mediators was observed after incubation in *p*-CouA and propolis. Additionally fungicidal and bactericidal activity of monocytes was improved (Cardoso et al. 2017). Incubation of human umbilical vein endothelial cells stimulated with lipopolysaccharides in *p*-CouA (10 μM) or red wine polyphenolic extracts (1–50 mg/L) resulted in lower endothelial inflammatory gene expression and reduced intracellular ROS generation, indicating that *p*-CouA have anti-atherosclerotic properties (Calabriso et al. 2016).

Application of cinnamon extract (containing approx. 0.25% of CinA) at concentration up to 100 mg/L to the coculture of human intestinal Caco-2 cells and macrophages ameliorated intestinal cell damage induced by lipopolysaccharide. Additionally treatment with cinnamon extract resulted in lower levels of inflammatory cytokines and could therefore be potentially useful in improving the symptoms of inflammatory bowel disease (Kim and Kim 2017).

23.4.6 Neuroprotective Effects

23.4.6.1 Animal Cell Cultures and Model Animals

Both *p*-CouA and *m*-CouA have neuroprotective effects in rat and mouse neuronal cell cultures. Incubation of rat neuronal cell lines in the presence of neurotoxic B-amyloid peptide, with 5–50 μM *p*-CouA, resulted in lower expression of proinflammatory enzymes cyclooxygenase-2 and inducible nitric oxide synthase (Yoon et al. 2014). *p*-CouA already at concentrations lower than 1 μM and also champagne wine extracts rich in *p*-CouA and other phenolic compounds protected mouse neurons in cell cultures from toxicity induced by 5-S-cysteinyl-dopamine (Vauzour et al. 2010). Feeding of mice with positional isomer of *p*-CouA, *m*-CouA (3 mmol/kg), which is a metabolite of chlorogenic acid, showed improved locomotor activity of mice, and further experiment on mice neurons showed that incorporation of 10 μM into growth media promotes neuronal differentiation and growth (Ito et al. 2008).

Main neuroprotective action of *p*-CouA is nevertheless related to the amelioration of the damages caused by anoxic conditions followed by restoration of blood circulation and oxygenation, which is accompanied by induction of oxidative stress (Szwajgier et al. 2017). The single intraperitoneal injection of *p*-CouA 100 mg/kg of body weight prior to induction of 20-min ischemia of the spinal cord followed by reperfusion resulted in significantly lower amount of formed malondialdehyde (marker of oxidative stress) and higher amount of antioxidant enzyme superoxide dismutase and respiratory factor 1 that activates expression of genes required for respiration. Additionally histopathological analysis of tissues reveals higher numbers of normal neurons 24 h after ischemia (Guven et al. 2015b). Similar effects of *p*-CouA were observed after ischemia/reperfusion of the sciatic nerve (longest nerve in the leg) (Guven et al. 2015c). Administration of 100 mg/kg of body weight

prior to occlusion of middle cerebral artery resulted in lower level of oxidative stress in ischemic brain tissue. Levels of malondialdehyde were decreased, whereas levels of superoxide dismutase and respiratory factor 1 were increased. *p*-CouA significantly improved neurological deficit scores (Guven et al. 2015a). The positive effects of *p*-CouA were observed also as a result of 2-week daily intake (100 mg/kg) together with normal diet. Mice fasted for 2 h prior to 30-min inclusion of both carotid arteries, followed by 45-min reperfusion, were sacrificed and their brains analyzed. Inclusion of *p*-CouA into the diet resulted in lower brain infarction volume and neuronal death. Similar to rats, content of malondialdehyde was decreased and level of antioxidant enzymes increased (Sakamula and Thong-asa 2018).

CinA has been shown to protect mouse dopaminergic neurons from damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and is therefore a potentially useful dietary molecule that could delay the onset of Parkinson's disease. Daily dose of CinA (100 mg/kg) fed to mice for a week improved locomotor activities most likely due to the activation of peroxisome proliferator-activated receptor alpha in astrocytes (Prorok et al. 2019).

23.4.7 Psychological Effects

23.4.7.1 Model Animals

Influences of *p*-CouA not only are limited to the neuroprotective function but also modulate learning and have anxiolytic (reduced anxiety) and antidepressant effects on model animals. Feeding of male Wistar rats with *p*-CouA (3–90 mg/kg) 60 min prior to testing significantly reduced anxiety. In vitro GABAergic assay revealed that *p*-CouA can bind to the receptor in micromolar range ($EC_{50} = 6.9$ μM), stronger than caffeic or cinnamic acids (Scheepens et al. 2014). Intraperitoneal administration of *p*-CouA (50–100 mg/kg) 1 day prior to behavioral experiments improved despair and apathy-related symptoms induced with lipopolysaccharide in rats. Biochemical analysis also revealed lower brain accumulation of proinflammatory cytokines cyclooxygenase-2 and TNF-a in the rats fed with *p*-CouA (Lee et al. 2018b). Rats with administered *p*-CouA (30 mg/kg) 30 min prior to injection of scopolamine, which impairs learning and memory, attenuated scopolamine-induced effects. *p*-CouA improved avoidance memory and long-term spatial recognition memory (Kim et al. 2017).

23.4.8 Bone Metabolism

23.4.8.1 Animal Cell Cultures, Model Animals, and Human Cell Cultures

Abnormal activation of osteoclast, which is normally involved in the process of bone remodeling, results in bone destruction in the diseases as rheumatoid arthritis. Incorporation of *p*-CouA into mannosylated liposomes (targeted to macrophages) injected into rats (10 mg/kg) resulted in significant inhibition of osteoclast differentiation and lower expression of inflammatory cytokines. Additionally incubation

of excised bones (with induced osteoclastogenesis) in the media containing 25 μM *p*-CouA resulted in significantly lower loss of bone calcium content (Neog and Rasool 2018). Oral administration of *p*-CouA 100 mg/kg for 10 days on the other hand significantly improves longitudinal bone growth in rats. Growth is most likely induced due to increased expression levels of insulin growth factor and growth hormone (Lee et al. 2018a).

Experiments on human osteoblastic cell cultures revealed that *p*-CouA already at submicromolar range increased proliferation of osteoblast for more than 10%. Induction effect was also observed when phenolic extract of olive oils containing *p*-CouA was added into the cell culture growth media (Garcia-Martinez et al. 2016). The influence on proliferation induced by *p*-CouA or phenolic extract was nevertheless not concentration dependent over the range of three orders of magnitude.

23.4.9 Hypoxia

23.4.9.1 Animal Cell Cultures and Model Animals

p-CouA is a quantitatively important constituent of Tibetan turnip that is traditionally used in local communities as food and folk medicine that ameliorates the symptoms of hypoxia. Experiments on model mice force-fed with *p*-CouA 100 mg/kg showed that symptoms of hypoxia (9.5% O_2 for 24 h) were significantly improved as less water was accumulated in lungs. On molecular level, concentration of NO was increased, redox status improved, and concentration of protein occludin that is crucial for alveolar stability increased (Li et al. 2018a). *p*-CouA (8 mg/kg administered daily for 1 week) was also shown to be efficient in improving the negative impacts of induced myocardial infarction in model mice as less edema and necrosis of heart tissue were observed. On molecular level, less oxidative damage was observed, and higher concentration of antioxidant enzymes was determined, which together with lower level of caspase expression resulted in lower degree of apoptosis (Prince and Roy 2013).

Interestingly *p*-CouA was also identified as a putative marker of oxidative stress per se. *p*-CouA was accumulated in human lobar bronchial cells, when cell culture was exposed to anoxic conditions. During 1-week incubation, an almost linear increase in *p*-CouA accumulation inside cells was observed. It is hypothesized that *p*-CouA is generated as a result of coenzyme Q10 degradation under anoxic conditions or downregulation of coenzyme Q10 biosynthesis for which *p*-CouA is precursor (Silina et al. 2016).

23.4.10 Eye Protection

23.4.10.1 Human Cell Cultures and Model Animals

Daily drops of *p*-CouA (164 ng) into the rabbit eye significantly reduced negative effect of UVB irradiation related to vessel damage. Concentration of marker typical for oxidative DNA damage (8-oxodG) was reduced, whereas concentration

of antioxidant enzyme superoxide dismutase was increased (Lodovici et al. 2009). Protective effect of *p*-CouA in the mixture with hydroxycaffeic acid (5 + 5 μM) was observed also in human conjunctival epithelial cell culture as synthesis of 8-oxodG was significantly reduced. Levels of proinflammatory prostangaldins and malondialdehyde that is formed as a result of lipid oxidation were reduced when daily dose (50 uL) at abovementioned concentration was administered to the rabbit eye (Larrosa et al. 2008). *p*-CouA (3–30 μM) included in growth media for human lens epithelial cells was protective against hydrogen peroxide-induced apoptosis. *p*-CouA downregulated expression of caspases and mitogen-activated protein kinases that were induced by hydrogen peroxide, suppressed intracellular ROS production, and increased concentration of antioxidant enzymes leading to lower level of cellular death (Peng et al. 2018).

23.4.11 Skin Pigmentation (Melanin Synthesis)

23.4.11.1 Animal Cell Cultures and Human Cell Cultures

Melanogenesis is the process of melanin formation in melanocytes that leads to skin pigmentation. Uncontrolled melanogenesis may result in undesired and aesthetically problematic skin pigmentation. Rice bran that is rich in *p*-CouA has been traditionally used for hypopigmentation treatment in East Asia. *p*-CouA and complex plant extracts with high content of *p*-CouA can interfere with melanin formation. Melanin formation and tyrosinase activity were significantly reduced already when *p*-CouA was added to murine melanoma cell culture at concentration lower than 10 mg/L. The inhibitory effect of *p*-CouA was much more pronounced than that of other structurally similar phenolic compounds (An et al. 2008). Further experiments revealed that inhibition of melanogenesis is the result of competitive inhibition of tyrosinase by *p*-CouA (Park et al. 2008) and that *p*-CouA is better inhibitor of human tyrosinase in comparison to those isolated from mice or mushrooms. Additionally *p*-CouA decreased expression of enzymes involved in melanogenesis due to inactivation of transcription factor (cAMP)-responsive element-binding protein (Jun et al. 2012).

23.4.12 Other Effects

23.4.12.1 Animal Cell Cultures, Model Animals, and Human Cell Cultures

p-CouA also influence some other physiological and pathophysiological processes. Administering a daily dose (50 mg/kg) to mice for 1 week significantly attenuated the hepatotoxic effect of acetaminophen, a popular antipyretic and analgesic drug, indicated by lower blood levels of aminotransferases. *p*-CouA protected from hepatic apoptosis, by attenuating ROS-mediated DNA modification and inflammation through the modulation of mitogen-activated protein kinase signaling (Cha et al. 2018). Negative effects of ethanol that induced reproductive toxicity in model rats

were suppressed by daily consumption of *p*-CouA (50–200 mg/kg). Antioxidant status, sperm parameters, and testis characteristics that were deteriorated by ethanol were improved by co-administration of *p*-CouA. Decreased level of pro-inflammatory molecules and apoptotic caspases was also observed (Nishi et al. 2018). Doxorubicin is an efficient chemotherapeutic yet cardiotoxic agent. *p*-CouA (380 μM) has a cardioprotective effect on cultured rat myoblast cells (from myocardium). The protective mechanism of *p*-CouA could be related to the upregulation of genes associated with autophagy that is crucial for the removal of nonfunctional organelles/proteins that are accumulated in cardiomyocytes as a result of doxorubicin treatment. Additionally *p*-CouA stabilized redox status and induced expression of antioxidant enzymes (Sunitha et al. 2018). *p*-CouA was also effective in preventing the oxidative damage of rat colonic mucosa. Daily administration (50 mg/kg) resulted in lower levels of 8-oxydG, better results of comet test, and induction of glutathione-S-transferase. Interestingly vitamin E administration of the same dose resulted in statistically significant increase of oxidative stress (Guglielmi et al. 2003). Oral administration of *p*-CouA (100 mg/kg per day for 3 weeks) proved to be efficient in protecting from cadmium-induced nephrotoxicity. Histopathological analysis of the rat kidney reveals that co-administration of *p*-CouA with cadmium halved the cadmium content in kidneys in improving their function. Higher content of metal-binding protein and improved redox status and lysosomal integrity were also observed (Luceri et al. 2007). Further experiments revealed that *p*-CouA attenuated synthesis of proinflammatory cytokines that were induced by cadmium and stabilized level of mitochondrial enzymes crucial for energy metabolism (Navaneethan and Rasool 2014).

p-CouA fed to rabbits at relatively low doses (5 mg/kg) inhibited ADP-induced platelet aggregation and thromboxane A2 (eicosanoid with prothrombotic properties), without negatively affecting other blood parameters (Luceri et al. 2007). Five consecutive administrations of *p*-CouA (20 mg/100 g) every third day significantly protected the spleen, liver, and bone marrow cells of mice from large radiation dose (5 Gy). Levels of blood serum aminotransferases and antioxidant enzyme that were changed as a result of irradiation were normalized in rats taking *p*-CouA. Positive effect were observed even 2 months after irradiation as lower levels of mitochondrial superoxide anions were detected in spleens and livers of mice that took *p*-CouA prior to exposure (Kook et al. 2017).

23.5 Benefits

p-CouA is on the crossroads of important biochemical pathways and can be formed by hydroxylation of trans-cinnamic acid or deamination of tyrosine by tyrosine ammonia lyase. It is the precursor of other cinnamic acids as caffeic, ferulic, and sinapic that are often present in food matrices at higher concentration than coumaric acid. There is large variety of soluble conjugates of cinnamates, including glycosides, ethers, and ethers of amides, depending on the compound that is bound to

hydroxyl or carboxylic group of particular cinnamic acid. Additionally cinnamates can be bound to the cell wall components with ether of ester bonds. Foods of plant origin are therefore a complex mixture of cinnamic acids and their derivatives. CouA and CinA (free or bound) and conjugates are rarely the predominant compounds. As no human nutrition studies with pure *p*-CouA or its conjugates were performed, data about benefits of *p*-CouA can be only extrapolated from the animal feeding studies (Sect. 4) that were performed mostly with rodents.

Despite lack of studies with pure compounds, there are some data obtained from dietary interventions with foods high in coumaric acid, to support findings on animal studies that food high in *p*-CouA and structurally similar compounds may have positive influence on human health.

In the randomized control studies, supplement of 50 g of oat flour per day in the duration of 8 weeks resulted in statistically significant higher potassium level and albumin, whereas some other markers of kidney function were not influenced by diet or even potentially negative factor as higher blood urea level was found in the "oat" group (Rouhani et al. 2018). Oat is a rich source of *p*-CouA and avenanthramides that include conjugates of *p*-CouA. The authors concluded that incorporation of oat into diet could be beneficial and speculate that some of the observed effects could be related *p*-CouA that also protects against nephrotoxicity in rats.

For Japanese apricot that is traditional food in Japan and is a rich source of *p*-CouA, it was shown that incorporation of this fruit into diet significantly reduce the level of allergic symptoms in rhinitis (Kono et al. 2018) especially in women. Within the same study, it was shown that oral administration of ume extract to mice attenuated mast cell degranulation led to lower response to allergen exposure. The same effect was also observed for the pure *p*-CouA.

Cinnamon as a spice has been used in human nutrition for centuries. There are a large number of tree species that are used as source for bark in which trans-cinnamic acid is one of the major constituents. Recently it was indicated that cinnamon consumption has beneficial effect related to diabetes due to lower blood glucose level. The systematic literature review published in 2016 (Costello et al. 2016) analyzed results of 11 randomized controlled trials. The conclusion was that consumption of cinnamon resulted in modest decrease of fasting glucose level as also lower level of hemoglobin glycation, indicating that it is a potentially useful supplement to standard therapy for type 2 diabetes. Additionally the randomized double-blind controlled trial on individuals with metabolic syndrome revealed that 3 g of cinnamon daily in the duration of 16 weeks resulted in significant improvements of markers of metabolic syndrome in the intervention group (Jain et al. 2017).

23.6 Application in Food

Food processing has large influence on the content and availability of polyphenols. Enzymatic and nonenzymatic oxidation, release of cell wall-bound polyphenols, de novo biosynthesis, leakage into the aqueous phase, and type of treatment are important factors.

23.6.1 Thermal Treatment

Parboiling of rice (five varieties) and bran from these varieties result in higher free and bound *p*-CouA content. Absolute increases in contents after parboiling were higher for the bran samples, whereas larger relative increase was found for milled rice (Pal et al. 2018). Thermal treatment of the sorghum bran (10 min boiling in water) prior to chewing or stimulation of gastric phase also resulted in improved bioaccessibility of *p*-CouA (Salazar-Lopez et al. 2018). By adding adzuki beans (20% w/w) to polished rice, the content of *p*-CouA can be increased. Availability of *p*-CouA from such mixture can be further increased, if cooked in high-pressure rice cooker in comparison to normal rice cooker (Woo et al. 2018). Higher availability of free *p*-CouA after high pressure cooking can on the other hand result in lower content of bound *p*-CouA. The content of bound *p*-CouA in cranberry beans that account for 80% of all *p*-CouA decreased for 40%, followed by only marginal increase of free *p*-CouA (Chen et al. 2015). Also thermal treatments of black rice bran at lower water contents like extrusion (120 °C, 12–17% moisture) can lead to higher free and bound p-CouA contents; nevertheless same treatment of polished and unpolished rice, where absolute p-CouA contents are lower, did not results in better availability (Ti et al. 2015b). Booth cooking and in vitro digestion positively affected availability (extractability) of cell wall-bound coumaric acid in brown rice that accounted for over 90% of all p-CouA. In polished rice, where initial p-CouA content is lower, cooking and digestion resulted in lower yields (Ti et al. 2015a). Booth cooking and in vitro digestion positively affected availability (extractability) of cell wall-bound coumaric acid in brown rice that accounted for over 90% of all *p*-CouA. In polished rice, where initial *p*-CouA content is lower, cooking and digestion resulted in lower yields (Ti et al. 2015). Thermal treatment at temperatures lower than 100 °C resulted in better retention of *p*-CouA in *Brassica* vegetables. The levels of *p*-CouA and some other phenolic acids were more stable in vegetables processed by sous-vide method (incubation at 90 °C, 45 min) in comparison to boiling and steaming (Florkiewicz et al. 2019). Microwave treatment (Zeb et al. 2019) significantly improved availability of *p*-CouA from chicory leaves. The fivefold increase of free *p*-CouA after 20 min of microwave treatment (650 W) was attributed to the release of cell wall-bound *p*-CouA. *p*-CouA is predominant phenolic in domestic garlic (Pedisic et al. 2018), and its content was reduced by crushing to the similar degree as blanching. Largest losses were observed for frying where only 40% of initial *p*-CouA remained after 10 min. Sorghum grains are excellent sources of free *p*-CouA (35 mg/kg), and processing like steaming and roasting results in substantial loss (up to 75%) of free *p*-CouA (Wu et al. 2013). Interestingly authors observed higher bound *p*-CouA, and such results could be interpreted more as an improved extractability due to processing as attributed to actual increase of bound *p*-CouA.

23.6.2 Sprouting and Fermentation

Sprouting and fermentation are relatively old techniques with renewed interest in relation to food processing and preparation. Here intensive transformation of matrix

takes place, which is accompanied by change in the content of bioactive constituents, as are phenolic compounds. Sprouting of wheat is accompanied by few-fold increase of free *p*-CouA content (few mg/kg range). Even higher contents are accumulated in the wheatgrass that is a popular garnish in high cuisine. Not only free but also the content of bound *p*-CouA that accounts for over 99% of *p*-CouA can be increased. Contents of bound *p*-CouA can be as high as 3500 mg/kg (Stagnari et al. 2017) that is more than order of magnitude higher than in the bran of cereals. Sprouted and kiln-dried wheat and barley malts are modest sources of free *p*-CouA (range of mg/kg) that can be extracted into beer. Here *p*-CouA serves as a precursor of 4-vinylphenol that is an important aroma compound in mature beer. Depending on the yeast strain, the *p*-CouA content in beer can be in the range of mg/L or can be decreased for the two orders of magnitude when yeast strains with high conversion potential are applied (Langos and Granvogl 2016). Free *p*-CouA content is increased also during germination of bean sprouts. For an order of magnitude, higher contents (200 mg/kg) were found in sprouted in comparison to raw beans. Accumulation of *p*-CouA and other polyphenols was significantly increased by elicitors of plant defense (Mendoza-Sanchez et al. 2016). In peanuts, where *p*-CouA is present in sub-mg/kg quantities and CinA in much higher content, (50 mg/kg) germination resulted in lower contents of both acids. Oven roasting and microwave heating also resulted in lower content of *p*-CouA and CinA (Aljuhaimi and Özcan 2018).

23.6.3 Other Treatments

Technological operations that result in change of structural integrity of plant matrix have also a large influence on available *p*-CouA. Preparation and roasting of cornflakes from flanking grits (large endosperm particles) result in decrease of bound *p*-CouA for all hybrids analyzed and three- to fourfold increase in soluble *p*-CouA. Total *p*-CouA content was nevertheless lower, as loss of bound *p*-CouA was not fully compensated by increase of soluble fraction (Butts-Wilmsmeyer et al. 2018). Defatted soybean flour contains few hundred mg/kg of bound *p*-CouA. Choosing the appropriate parameters of microwave or ultrasound treatment can result in the release of bound *p*-CouA, and soluble content can reach almost 100 mg/kg, which accounts for 20% of initially bound *p*-CouA (Jez et al. 2018). High-pressure treatment (up to 650 MPa) on the other hand did not result in higher soluble *p*-CouA content in tomato puree. For all treatments and varieties, even losses of *p*-CouA up to 40% were observed (Durovic et al. 2018). Similar was found for mulberry juice where ultrahigh pressure processing resulted in almost 50% loss from initial 80 mg/L. Addition of ascorbic acid into the juice prior to processing resulted in complete retention of *p*-CouA indicating that oxidation is possible mechanism of loss (Yu et al. 2014). Prolonged storage of tomato juice results in loss of *p*-CouA and other phenolic compounds. It was nevertheless shown that moderate-intensity pulsed electric field treatment of juice prior to storage improves initial content and retention of *p*-CouA during storage (Vallverdu-Queralt et al. 2012).

23.7 Toxicity and Side Effects

In principle anticancer properties of coumaric acid, as increased production of ROS, induction of caspases and apoptosis, and lower expression of enzymes involved in detoxification pathways, can be in broader terms related to toxicity and undoubtedly result in side effects. Despite the abovementioned cytotoxic effects, the acute toxicity of *p*-coumaric acid is relatively low (LD_{50} for mice = 2850 mg/kg). The content of free-*p*-CouA in food of plant origin is rarely above 100 mg/kg DW and in certain edible mushrooms in the order of 1000 mg/kg DW. Glycosylated forms (conjugates) in certain berries reach 1000 mg/kg DW range. The highest contents are found for cell-bound *p*-coumaric acid and in not edible parts as the corn straw and cob or residue of sugarcane extraction (bagasse) where the contents can be as high as 20,000 mg/kg of DW. The bioavailability of such *p*-CouA is much lower, and the kinetics of the release in gastrointestinal tract relatively slow. Therefore, with normal daily nutrition, it is hard to obtain doses that are even for two orders of magnitude lower than LD_{50} found for mice.

One of the important characteristics of *p*-CouA is that influence on ROS formation significantly depends on concentration and can attenuate or stimulate the level of oxidative stress. Incorporation of 0.5 μM *p*-CouA into growth media for human endothelial cells resulted in statistically significant lower levels of ROS and increased metabolic activity, whereas at 20-fold higher concentration (10 μM) formation of ROS was increased and cell viability decreased (Posadino et al. 2013). Experiments on rat liver mitochondria revealed that *p*-CouA inhibit pyruvate transport and carboxylation and in sub-100 μM range inhibit certain enzymes involved in gluconeogenesis (Lima et al. 2006). This is the concentration range of *p*-CouA that can be found in plasma of rodents few minutes after oral ingestion of doses around 100 μmol/kg (Pei et al. 2016). The half-life of *p*-CouA in plasma is nevertheless short (range of minutes), and therefore the concentration is relatively fast decreased to low micromolar range. Potentially the most problematic effect of *p*-CouA is its goitrogenic activity (Khelifi-Touhami et al. 2003). The mass of the thyroid glands of rats with ad libitum access to standard laboratory chow with sufficient level of iodine that received by gastric tube a 40 mg/kg of *p*-CouA for 3 weeks was doubled in comparison to control. The goitrogenic effect was confirmed by a significant decrease of triiodothyronine and thyroxine hormones in blood. Importantly the goitrogenic effect of *p*-CouA was much more pronounced than that of caffeic and ferulic acids fed in the same amount (based on molar intake). No further experiments related to potential goitrogenic effect at lower doses in rodents of human intervention studies were performed.

p-CouA is much more toxic toward certain bacteria and fungi than toward mammals. Antimicrobial action of *p*-CouA can therefore be used for stabilization of food matrix in order to reduce microbial load. In the millimolar range, *p*-CouA can completely inhibit *Staphylococcus aureus* growth in the chicken soup even at room temperature (Stojkovic et al. 2013). In submillimolar range, *p*-CouA significantly increased the permeability of bacterial cell membrane and also intercalated the groove in bacterial DNA double helix, which might affect DNA replication and

transcription (Lou et al. 2012). *p*-CouA showed a synergistic antibacterial effect against both gram-positive and gram-negative bacteria with nisin (Bag and Chattopadhyay 2017), antibacterial peptide that is used as a food additive. The mechanism of action is most likely related to the inhibition of surface attachment and biofilm formation. Combination of nisin and *p*-CouA could therefore find the application in the food industry. Lower microbial loads can also be related to quorum sensing inhibition. The statistically significant effect was nevertheless observed at 3 mmol/L or higher *p*-CouA concentration, which limits practical application (Bodini et al. 2009). Analysis of mechanisms of tolerance studied on bacterium *Pseudomonas putida* revealed that improved membrane stability, secretion, and exclusion of toxic compounds outside the cell were crucial for survival (Calero et al. 2018).

CinA is a potent inhibitor of quorum sensing and potential antivirulence agent for control of bacterial infection. At sublethal concentration, it effectively inhibited biofilm development and production of quorum sensing-dependent virulence factor of *Pseudomonas aeruginosa*. Modeling in silico and in vivo analysis on *C. elegans* revealed that CinA interferes with the binding of natural autoinducers to the quorum-sensing receptor proteins (Rajkumari et al. 2018). Aquaculture industry is under a large pressure to find the alternative to the application of antibiotics that are undesired pollutants. It was shown that CinA has potent inhibitory effect (mg/mL range) against certain fish pathogens, whereas the majority of nonpathogenic isolates from fish intestines were resistant to trans-CinA (Yilmaz et al. 2018). Photochemical isomerization of trans-CinA to cis-CinA have large influence on antimicrobial activity. The minimum bactericidal concentration of cis-CinA against multiple drug-resistant *Mycobacterium tuberculosis* is as low as 2.5 mg/L, which is more than two orders of magnitude lower than that of trans isomer (Chen et al. 2011).

Viability of fungi is also influenced by *p*-CouA and its derivatives. Ethanolic ester of *p*-CouA at 1 mmol/L increased plasma membrane permeability and induced oxidative damage of cellular structures of fungus *Alternaria alternata* that can produce mycotoxins on the surface of fruits and vegetables (Li et al. 2018b). Acidic and relatively nonpolar *p*-CouA can diffuse through the cellular membranes. In phytopathogenic fungus *Botrytis cinerea*, *p*-CouA (1.5 mM concentration in growth media) can act as a mitochondrial uncoupler of proton gradient (Morales et al. 2017), resulting in the increase of oxidative metabolism, accompanied by relatively low ATP synthesis, offering the alternative way for the control of this pest, that can significantly affect yield and quality of grapes.

Structural isomer of *p*-CouA, the *o*-CouA, is a potent phytotoxin. Already in the submillimolar concentrations, it reduced the germination of *Arabidopsis* seeds, inhibited root elongation, and induced cell death, whereas other two isomers, *p*-CouA and *m*-CouA, had no effect in this concentration range. It is hypnotized that *o*-CouA can play the important role in the spreading of the invasive plant *Eupatorium adenophorum*, where *p*-CouA is a major phenolic compound (Zheng et al. 2012).

p-CouA can be used as an antimicrobial agent also in human body ecosystems. Adhesion of oral pathogens to the cells of connective tissue was significantly

inhibited by *p*-CouA already at concentrations lower than 100 μmol/L (Esteban-Fernandez et al. 2018). Importantly most of bacterial pathogens found in oral cavity do not metabolize *p*-CouA, and its concentration remains relatively stable.

23.8 Marketed Product

In the recent years, a lot of efforts have been put into the research areas related to the study of influence of various processing and cooking parameters on the bioavailability of micronutrients, including coumaric acid. This research effort will undoubtedly result in the marketing of food products with optimized content of such bioactive constituents. With the normal diet, milligram quantities of positional isomers of coumaric acid are consumed on a daily basis. Apart from food, coumaric acids can be consumed with dietary supplements, where they contribute to the functional and health-related properties of the products. For vast majority of such products, detailed composition of micronutrients is not reported, and therefore the actual impact of coumaric acids to the characteristics of the product can be only estimated from scientific sources where composition of phenolic compounds in similar matrices was determined. For some products that are advertised on the Web, actual content of *p*-CouA is nevertheless reported. The propolis extract enriched with astaxanthin (https://www.naturalhealthyconcepts.com/dr-ohhiras-propolis-plus-30.html) contains 730 μg of *p*-CouA/g or 1320 μg in a serving (three capsules). There are also scientific manuscripts reporting the compositions of particular dietary supplements. Certain *Vitis vinifera* extracts that are sold on the EU market contain 100–600 ug/g of *p*-CouA, which in particular supplements also the major phenolic compound (Filipiak-Szok et al. 2013). Potential health-related benefits of such extracts could be related to *p*-CouA. Dietary supplement prepared from *Moringa oleifera* seeds by fermentation, which contains 3000 ug of *p*-CouA/g, modulates HDL and LDL cholesterol ratio, and has immunological properties (Adedayo et al. 2018), is a promising product with market potential. Application of *p*-CouA is undoubtedly not limited only to the food and dietary supplement sector. Due to antityrosinase activity (prevents skin pigmentation), it is one of the most important ingredients of products with cosmeceutical application (Taofiq et al. 2017).

Cinnamon bark, pulvers, or extracts are used as spices and dietary supplements. Alcoholic extracts of cinnamon are available from various suppliers mostly as food-flavoring products. Dried extracts are often packed into gelatin capsules and sold as dietary supplements. They are advertised as products that support insulin function and maintain optimal glucose level. The actual composition of the extracts is typically not reported, and therefore the efficiency of the products cannot be related to the experimental results published in scientific literature that were obtained with chemically defined samples. Large variations in the bioactive constituents in different cinnamon species and type of solvents used for extraction result in large variations in the composition of extracts (Costello et al. 2016).

23.9 Patents

As already mentioned in the previous section, *p*-CouA and its derivatives have a large potential as antipigmentation agent for the skin. In relation to this property, in the Google Patents base (https://patents.google.com/), patents in the USA (US9089499B2) and elsewhere were issued. *p*-CouA and other CinA derivatives are also the compounds with two functional groups, which can form ester bonds, and are as such appropriate compounds for polymerization reaction and formation of bioplastics. The patent related to formation of *p*-CouA-based polymers (US20190002630) was issued in 2019. *p*-CouA is abundantly available from non-food agricultural wastes as bagasse or corn stover. Plastics made from CinAs could be interesting for packaging of food items due to antioxidant properties based on hydroxyl groups on aromatic rings. Apart from agricultural waste products, *p*-CouA can be obtained also biotechnologically by deamination of tyrosine. In 2016 patent (WO/2016/008886) related to microbial transformation based on tyrosine ammonia lyase activity was published.

One of the patents related to CouA has also important implication in food products. In the recent years, there is a large interest for reduction of sugar content in food products. In order to retain sweet taste in food with reduced sugar content, artificial sweeteners or steviol glycosides from plant extracts can be added. Sweet taste of sugar substitutes is nevertheless different from that of mono- and disaccharides. Often a slightly bitter aftertaste, metallic, and even rancid impressions in the oral cavity are produced. Certain substances can mask this aftertaste and improve the acceptance of sweet products with low sugar content. The EU patent "Aroma composition comprising *o*-coumaric acid to reduce or suppress undesirable taste impressions of sweeteners" (EP2340719B1) related to the application of *o*-CouA as compound that can modify the unpleasant aftertaste of non-sugar sweeteners was granted in 2014.

Antimicrobial activity of cinnamic acid forms the basis for few patents that were issued in relation to its application as preservative in nonalcoholic beverages. The Japanese patent (JP4267814B2) granted in 2009 is related to the use of Cinn as a preservative in tea-based drinks that could be prepared in the process of cold filling. The European patent (EP2768326B1) related to the application of vanillin and CinA as preservative in carbonated and juice-containing beverages was granted in 2018.

23.10 Perspectives

Due to the advanced analytical techniques, there is an increasing amount of data of polyphenolic composition of foods, metabolic products, and their pharmacokinetic effects. The data are becoming unmanageable, so there is a need for up-to-date databases with required deposition of the new data. There is a need for standardization of analytical methods and reference materials so that the data from different laboratories can be compared. For some classes of dietary polyphenols, there are

now sufficient intervention studies to indicate the type and magnitude of effects among humans in vivo, on the basis of short-term changes in biomarkers. However, a large number of polyphenolics remain unexplored with respect to their antioxidant and other human beneficial properties. Furthermore, substantial evidence is needed to elucidate the molecular mechanisms of different classes of phenolic compounds involved in the potential protective effects against oxidative stress. HCAs have significant antioxidative activity, anti-inflammatory, anticancer, and many other beneficial roles. To follow their activity in vivo, there is a need for plasma biomarkers (for the antioxidative activity, energy metabolism, carcinogenesis). The effects of phenolic acids including *p*-CouA and CinA (found in raw and processed foods such as red wine, cereals, cranberries, and some supplements) are mostly studied in vitro; knowledge about the effects in vivo, although they are significant, is more limited. The reasons are (i) the lack of validated in vivo biomarkers, especially in the area of carcinogenesis; (ii) lack of long-term studies; and (iii) lack of understanding or consideration of bioavailability in in vitro studies, which are subsequently used for the design of in vivo experiments. It is time to rethink the design of in vitro and in vivo studies, so that these issues are carefully considered. To more closely reflect the long-term dietary consumption of polyphenols, the length of human intervention studies should be increased.

23.11 Cross-References

- ▶ Antioxidants in Diets and Food
- ▶ Caffeoylquinic Acids
- ▶ Chlorogenic, Caffeic, and Ferulic Acids and Their Derivatives in Foods
- ▶ Dietary Coumarins
- ▶ Stilbenoids in Grapes and Wine

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Dietary Ellagitannins

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Contents

Abstract

Ellagitannins (ETs) are widely distributed secondary metabolites classified as hydrolyzable tannins found mainly in berries such as raspberries, blueberries (BBs), black raspberries (BRBs), strawberries, and pomegranates. A plethora of reports tend to show that ETs can impact positively on human health by

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promoting therapeutic activities such as being an antioxidant, antimicrobial, anti-inflammatory, and anticancer. Studies showed that ETs found in the seeds of black raspberries (BRBs) increase the functional activity of juices or wines which used BRBs as ingredients. It has been reported that berries have the potential to reduce the systolic and diastolic blood pressure and increase superoxide dismutase activity after the consumption of BBs. Numerous studies showed that ETs isolated from pomegranates possess good antimicrobial activities against a panel of microbes including human pathogens such as *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Candida albicans*. Interestingly, it was shown that these therapeutic values are attributed to the presence of ETs. Furthermore, ETs are also distributed in various nuts, namely, pecans, cashews, walnuts, pistachios, or hazelnuts. It is known clinically that frequent nut intake is associated with protective effects against cardiovascular diseases. Although it is mentioned that ETs can also be found in vegetables, however as far as our literature search could establish, there are no scientific validation of ETs in vegetables. Thus, it is proposed that more scientific investigations be geared to shed light on the potential of ETs found in vegetables. Overall, foods containing ETs are recommended to be consumed on a regular basis since a panoply of scientific investigations showed that they are associated with promising health benefits.

Keywords

Ellagitannins · Black raspberries · Vegetables · Pomegranates · Nuts · Health benefits

24.1 Introduction

Phytochemicals are non-nutritive secondary plant compounds often with health-promoting and disease-preventive properties, mainly found in fruits, vegetables, grains, herbs, spices, and other plant foods (Afrin et al. 2018). For many decades ago till date, plants have been the center of interest for many scientists across the globe solely due to their curative nature. The therapeutic value of edible plants and fruits is linked to the metabolites they produced and has indeed proved their worth on many occasions. For instance, biguanide compounds present in various plant parts such as dry fenugreek seeds (*Trigonella foenum-graecum* L.), fresh green curry leaves (*Murraya koenigii* L.), fresh green bitter gourd (*Momordica charantia* L.), garlic (*Allium sativum* L.), orange-fleshed sweet potato (*Ipomoea batatas* L.), and white-fleshed potatoes (*Solanum tuberosum* L.) exhibited good insulin sensitivity and thus control glucose level significantly (Raigond et al. 2018; Perla and Jayanty 2013). Plants are considered as a major storehouse of metabolites production of various phytochemical classes. It is found that phenolic contents or polyphenols are the most ubiquitous metabolites widely distributed in the kingdom *Plantae*, and the

most common polyphenols include phenolic acids, tannins, and flavonoids (Sytar et al. 2018; Ksouda et al. 2018).

Polyphenols form the integral part of most human diets and even animal diets since many among them are herbivores (Ovaskainen et al. 2008). Recent interest in food phenolics has increased due to their role as antioxidants which act as a prevention barrier against numerous diseases, namely, cardiovascular diseases, cancer, Alzheimer's disease, and age-related diseases (Basanta et al. 2018; Bravo 1998). Tannins are medium-sized phenolic compounds of up to 30,000 Da. They are subdivided into two major categories: (1) hydrolyzable and (2) condensed tannins. Additionally, a third minor group of tannins exist known as phlorotannins usually found in marine brown algae but are not edible (Bravo 1998). Hydrolyzable tannins, as their names indicate, are readily hydrolyzed in weak acid, alkali, or hot water to form polyhydric alcohol and phenylcarboxylic acid (Shirmohammadli et al. 2018). Based on this chemical property, hydrolyzable tannins are further subdivided into two groups: (1) gallotannins (released gallic acid) and (2) ellagitannins (released ellagic acids) (Shirmohammadli et al. 2018; Bravo 1998) (Fig. 1). Ellagitannins (ETs) are hydrolyzed to ellagic acids (EAs) under physiological conditions in vivo, and the latter is then metabolized by the microbiota in the intestine to produce an array of different urolithins such as urolithins A and B (Sánchez-González et al. 2014; Landete 2011).

ET is characterized by the presence of a unit, biarylic dehydrodigalloyl so-called hexahydroxydiphenoyl (HHDP), often linked to 1,2,3,4,6-hydroxy of glucose (Yang et al. 2018). In 1996, the first total synthesis of ellagitannin was identified as pedunculagin (Fig. 2) and was performed by Feldman and Smith in the Pennsylvania State University.

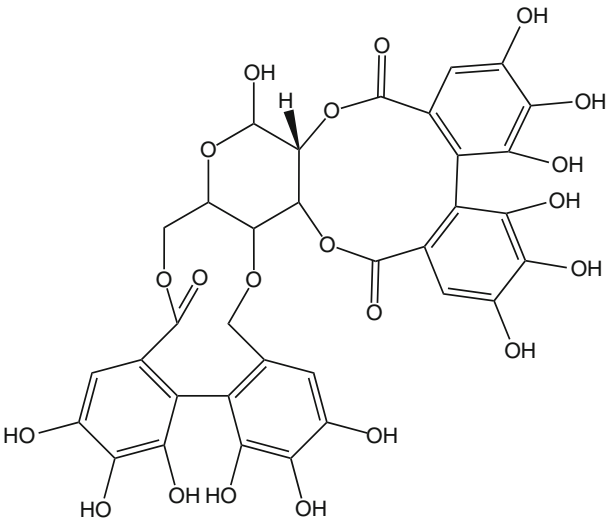


Fig. 1 Pedunculagin

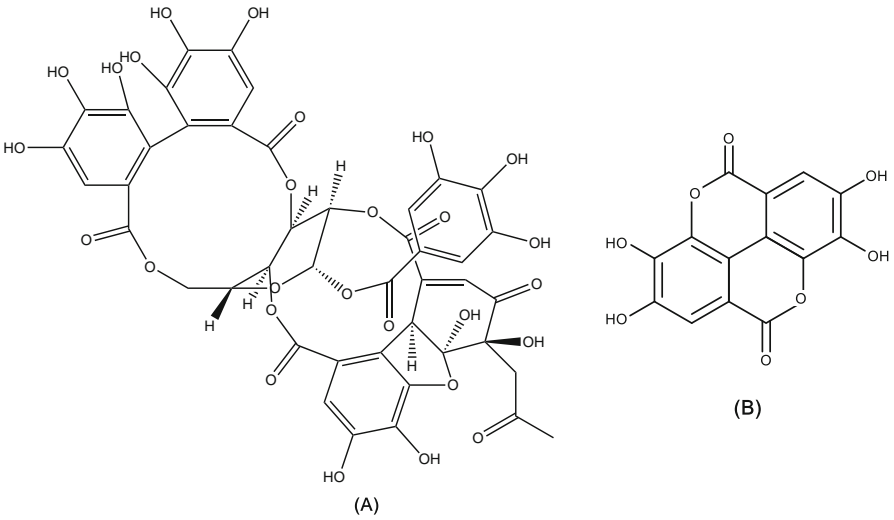


Fig. 2 (**a**) Ellagitannin, (**b**) ellagic acid

During the past decades, researches were mainly focused on phenolics, flavonoids, and anthocyanins but less on ETs. The reason was linked to the occurrence of ETs, and EAs are mostly found in fruits, few berries, and nuts (Törrönen 2009). Numerous vegetables and fruits possessed many therapeutic values due to their high consistency of a variety of phytochemicals. The most important dietary phytochemicals considered are isothiocyanates, indoles, flavonoids, retinoids, tocopherols, and ellagitannins (ETs) (Ismail et al. 2016). Various studies have confirmed that diets rich in fruits and vegetables reduce the risk of many types of cancer, namely, colorectal cancer (Afrin et al. 2018; Bansal et al. 2018), breast cancer (Sayeed et al. 2017), lung cancer (Kasala et al. 2015), and skin cancer (Iqbal et al. 2019) and also neurodegenerative diseases (de Rus Jacquet et al. 2017).

24.2 Bioactive Constituents

Ellagitannins are a type of polymer formed by EA and a hydroxyl group, like glucose, bind together (Fig. 2). They are characterized by the presence of biarylic dehydrodigalloyl so-called hexahydroxydiphenoyl (HHDP) units which are frequently linked to 1,2,3,4,6-hydroxy of glucose. As a diverse class of hydrolyzable tannins, ETs are different from gallotannins, which are characterized by the presence of depsidically linked galloyl units. Ellagitannins generally form macrocycles, while gallotannins do not. Considered a type of antioxidant, ETs can be found in various fruits. Some of them exhibit remarkable bioactivities as discussed in the next sections of this chapter, such as potent antibacterial and antiviral activities, inhibition of mutagenicity of carcinogens and tumor promotion, as well as remarkable host-mediated antitumor effects (Yang et al. 2018).

24.3 Bioavailability and Metabolism

Ellagitannins are polyphenols that possessed significant biological activities in vitro and relevant pharmacological properties and nutritional values in vivo. The promising biological activities are related to the scavenging ability that ETs possessed. Nevertheless, it is equally important to understand the bioavailability and metabolism of ETs in the gastrointestinal (GI) tract to understand the physiological or nutritional role of these polyphenols.

Ellagitannins and ellagic acids are found abundantly in fruits, namely, pomegranates, berries, peaches, and cherries, vegetables, and nuts such as walnuts, cashews, and pecans (Landete 2011). A fresh weight of 100 g of raspberries can serve approximately 263–330 mg ETs, eight walnuts can provide an amount of 802 mg, a liter of pomegranate juice contains 2020–2660 mg, and a liter of oak-aged red wine contains about 9.4–50 mg of ETs. Table 1 illustrates the amount of ETs and EAs recorded from different types of foods. The amount of EAs in foods is determined as free EA or

Table 1 Contents of ETs and EAs in foods

Food	Content	References
Fresh fruits:		
Raspberry	263–330 mg/100 g fw	Koponen et al. (2007)
Strawberry	77–85 mg/100 g fw	
Strawberry Blackberry Pomegranate Muscadine grape Navel orange Peach Red grape Kiwi Cranberry	25 mg/100 g fw 1.5–2.0 mg/ g dw 35–75 mg/100 g fw 36–91 mg/100 g fw <100 μg EA/mg dw <100 μg EA/mg dw <100 μg EA/mg dw <100 μg EA/mg dw <100 μg EA/mg dw	Aaby et al. (2007) Clifford and Scalbert (2000) Gil et al. (2000) Törrönen (2009) Daniel et al. (1989)
Nuts:		
Walnut	802 mg/50 g (8 nuts)	Anderson et al. (2001)
Pecan	590 ± 1 μg EA/mg dw	Daniel et al. (1989)
	20.96–86.2 mg/g	Malik et al. (2009)
	330 ± 0.3 μg EA/mg dw	Daniel et al. (1989)
Cashew	<100 μg EA/mg dw	Daniel et al. (1989)
Processed fruits:		
Pomegranate juice Raspberry juice Raspberry jam	2020–2660 mg/L 76 mg/100 g fw 24 mg/100 g fw	Gil et al. (2000) Koponen et al. (2007)
Wines:		
Oak-aged red wine	9.4 mg/L	Glabasnia and Hofmann (2006)
	50 mg/L	Clifford and Scalbert (2000)
Spirits:		
Whiskey	1–2 mg/L	Glabasnia and Hofmann (2006)
Cognac	31–55 mg/L	Clifford and Scalbert (2000)

fw fresh weight, *dw* dry weight, *EA* ellagic acid

total EA after acid hydrolysis (Landete 2011). High content of ETs and EAs is reported in mainly all of the berries (strawberries, raspberries, blackberries) (Koponen et al. 2007; Aaby et al. 2007; Clifford and Scalbert 2000) as shown in Table 1.

Pomegranate juice is a rich source of ETs and exerts a protective property against a panel of diseases especially cardiovascular diseases, hypertension, blood lipid profile, and oxidative stress. Pomegranate juice was used for 3 years to treat patients suffering from carotid artery stenosis which may lead to decreased atherosclerotic lesion size. This effect is attributed to potent antioxidant ability of pomegranate juice which contains large amount of ETs. Bianca Fuhrman (2006) reported a decline in collagen-induced platelet aggregation in patients after they consumed pomegranate juice for 2 weeks.

Dietary foodstuffs containing ETs and EAs have promising anticancer property. However, for research purposes, it is important to know the bioavailability and metabolism of these polyphenols for a better selection of cell lines. For example, if ETs and EAs are poorly absorbed from the gut, it is then inappropriate to test these polyphenols at very low concentrations. For instance, a large amount of the tested compounds would be needed to reach the target organs (e.g., breast, brain). Numerous studies have demonstrated the significant anticancer property of ETs found in pomegranates, raspberries, and strawberries among others against different types of cancer.

Generally, ETs are relatively stable in the physiological conditions of the stomach. Acidic conditions (pH 1.8–2.0) and enzymes do not hydrolyze ETs, and thus no EA formation is observed in the stomach. However, under the physiological conditions of the stomach, a small amount of EAs is converted from ETs due to the physiological conditions with pH 7.0–7.3 (neutral conditions). Several human gastrointestinal (GI) cell lines are normally used to evaluate the uptake, metabolism, transport, and excretion of ETs and EAs. Numerous studies used predigested ETs (Caco-2 cells) for a close simulation of the in vivo conditions. Whitley et al. (2003) reported a high accumulation of EA in Caco-2 cells giving an indication that EAs are easily absorbed across the apical membrane. Around 93% of EAs are irreversibly bound to macromolecules; hence it appears to accumulate in the epithelial cells in the aerodigestive tract.

ET and EA are metabolized further by the intestinal microflora to form urolithins A and B. Cytochrome P-450 hydrolyzed urolithin A to increase the possibilities of glucuronidation and consequently increase the excretion of the metabolite. Two experiments have been carried out to understand the bioavailability and absorption of ETs in the human body. Firstly, the pharmacokinetic of the absorption of pomegranate juice is studied for several hours after consumption, and secondly the bioavailability and metabolism is evaluated by studying the metabolites present in the urine and plasma samples. Seeram et al. (2004) reported a concentration of 31.9 ng/mL of EA in plasma in the first 4 h after consumption. A second study investigating on ET bioavailability and metabolism was carried out with other dietary source of ET, namely, strawberry, raspberry, walnut, and oak-aged red wine (Cerda et al. 2005). These foodstuffs differ in the content and type of ETs. However, urolithin A was detected in all participants ($n = 40$). This led to the proposal of the microbial metabolite urolithin A as a biomarker for human exposure to dietary ETs.

Ellagitannins are large polar polyphenols poorly absorbed in the bodily system. Some pharmacokinetic studies show that EA can be absorbed within 30–60 min after the intake of several foods (Seeram et al. 2004). This indicates that absorption of EAs starts in the stomach and is detectable in peripheral blood. Generally, EAs disappear from plasma 2 h after consumption. Nevertheless, several pharmacokinetics have shown that after the intake of different foods containing the same amount of EAs, no apparent detection of EA was observed in plasma indicating that no absorption of EA took place. However, it is important to note that different individuals have different absorption pattern.

One of the most important steps in bioavailability and metabolism of ETs is the conversion of ET into different hydroxylated dibenzopyranone derivatives, namely, urolithins A–D. Urolithins are the gut microbial metabolites of ETs. The conversion starts in the small intestine and continues in the GI tract. Urolithins A and B are the two most important types of hydroxylated dibenzopyranone derivatives. Urolithins are absorbed in the small intestine and excreted in the bile after methylation and conjugation with glucuronic acid (Espin et al. 2007). Experimental testing conducted in animals showed that the reason why the formation of these metabolites starts in the small intestine could be due to the presence of anaerobic bacteria. The metabolism continues along the GI tract to end with the production of urolithins A and B. Differences in the production of these metabolites by human volunteers show that they may be produced by the activity of specific microorganisms present in the gut. If these microbial metabolites, which are more bioavailable than native ETs and EA, were the true active principles responsible for the biological activity associated with ET- and EA-rich foods consumption, then this would bring up the possibility of developing new functional foods in which specific ET-metabolizing microorganisms could be included together with the ETs (Fig. 3).

Furthermore, in the GI tract and in other tissues (mainly in the liver), EA and ETs microbial metabolites are further metabolized either through Phase I (hydroxylation) or Phase II (methylation, glucuronidation, and sulfation) enzymes to render them more soluble for better excretion in urine. Hydroxylation of urolithin A, and probably B, renders them more reactive with additional

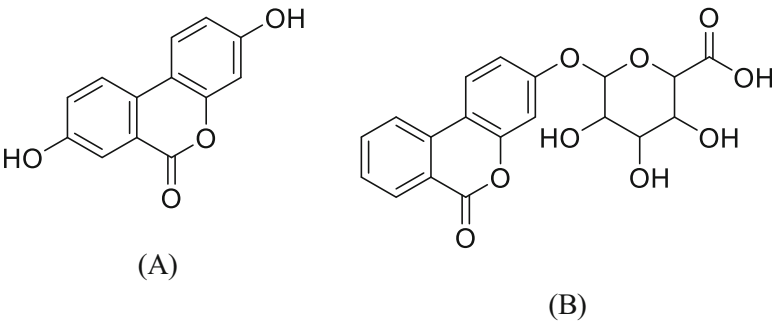


Fig. 3 (**a**) Urolithin A, (**b**) urolithin B 3-*O*-glucuronide

functions for conjugation, which hence facilitates their excretion. Thus, monohydroxydibenzopyran-6-one (urolithin B) can be hydroxylated to produce a dihydroxylated derivative, and urolithin A (3,8-dihydroxydibenzopyran-6-one) can be further hydroxylated to produce trihydroxylated derivatives (Quideau 2009). A study showed that both urolithins A and B can induce the expression of cytochrome P-450 in Caco-2 cells by 15- and 20-fold explaining the abundance of urolithin B-derived dihydroxylated compound in urine, plasma, and liver tissues. Phase II products are also produced together with methyl ethers and different glucuronide conjugates and are detected in different tissues and in urine. The sulfate conjugates of the metabolites of ETs are more abundant in animals and humans compared to glucuronide conjugates. These sulfate conjugates are initially produced in the intestinal cells before being further metabolized in the liver and finally excreted in urine or bile.

In order to understand the biological activity of ETs and EA, it is important to know the metabolites present and their respective concentrations in the target organs. For instance, in rats, no EA, ET, or derived metabolites have been detected in muscle, adipose, heart, lung, or brain tissues. However, a minute amount of microbial metabolites were detected in the liver and kidney. The biological activity and physiological effects of ETs and EA have to be consistent with their bioavailability and metabolism. Thus, results obtained from in vitro studies using cultured cells that would not be in direct contact in vivo with food ETs should be considered with caution.

24.4 Bioactivities (Animal Aspects)

There are also a number of reports regarding the in vivo chemopreventive activity of EA and ETs, mostly in animal models. These studies started in the early 1960s, when it was shown that EA was able to inhibit induced lung tumors in mice upon oral administration. It has been observed that EA and ETs can disappear when in contact with animal cells or tissues, and this has been attributed to irreversible linkage of these compounds with proteins or other cell structures. The bioavailability studies of EAs in animals showed that EA is released from ET in the small intestine followed by absorption of EA in the cells. EA is then easily metabolized in the intestinal cells to form methyl and dimethyl ethers and glucuronic acid conjugates. These metabolites are detectable in bile and in peripheral blood; however no free EA was detected (Quideau 2009).

Prostate cancer is the second leading cause of cancer among men across the globe. Numerous studies suggested pomegranate juice as good cancer-chemopreventive as well as cancer-chemotherapeutic agents against prostate cancer. A study conducted by Malik et al. (2009) mentioned that anthocyanins present in pomegranate juice are responsible for the anticancer effect in athymic mice. Two different groups of mice were administered with pomegranate fruit extract (PFE) as and water as drinking fluids, respectively. Eight days after consumption, it was observed that small solid tumors were formed in mice drinking water. PFE in

different percentages, namely, 0.1% and 0.2%, were administered orally in mice 1 day after implanting tumor cells. It was observed that both 0.1% and 0.2% PFE significantly inhibited tumor growth.

24.5 Benefits (Human Studies)

Consumption of dietary ETs has been associated with different health benefits. Nonetheless, ETs are not bioavailable as such and are metabolized in vivo. They are partially converted into EA in the upper gastrointestinal (GI) tract, but this first metabolite is also poorly bioavailable. In the lower GI tract, EA and residual ETs are metabolized by gut microbiota to produce urolithins, which, together with their conjugate relatives, persist at relatively high concentrations in plasma and urine for days after ingestion of dietary ETs. Thus, ETs and EA may exert local health benefits on the GI tract, but systemic health benefits are more likely to result from urolithins (Garcia-Munoz and Vaillant 2014). Many beneficial effects are related to the presence of EA, ETs (including punicalagins), punicic acid and other fatty acids, flavonoids, anthocyanidins, anthocyanins, estrogenic flavonols, and flavones, which seem to be its most therapeutically beneficial components. However, the synergistic action of the pomegranate constituents appears to be superior when compared to individual constituents. Promising results have been obtained for the treatment of certain diseases including obesity, insulin resistance, intestinal inflammation, and cancer. Although moderate consumption of pomegranate does not result in adverse effects, future studies are needed to assess safety and potential interactions with drugs that may alter the bioavailability of bioactive constituents of pomegranate as well as drugs (Viladomiu et al. 2013).

24.6 Pharmacological Properties of Dietary Ellagitannins

Numerous food products, medicinal plants, and nutraceuticals contain hydrolyzable tannins mainly ETs which are known to possess potential benefits on cardiovascular diseases (Larrosa et al. 2010). From a study reported by McDougall and Stewart in 2005, it was mentioned that ETs inhibited α-amylase activity and thus have the potential to show synergistic effects on starch degradation after ingesting berries particularly raspberries and strawberries (McDougall and Stewart 2005). Additionally, a study conducted by Ismail et al. (2016) showed that ETs play a primordial role in the prevention of cancers, namely, cervical, skin, liver, colon, breast, prostate, and esophageal and oral cancers. Ellagitannins are found in abundance in fruits mainly raspberries, strawberries, pomegranates, blackberries, and longan and nuts especially almonds and walnuts (Heber 2011). Interest in ETs and EA has increased over the past few years due to its properties as a micronutrient (Bakkalbaşi et al. 2008).

Bioactive compounds from berries have potent antioxidant, anticancer, antimutagenic, antimicrobial, anti-inflammatory, anti-neurodegenerative (Nile and

Park 2014), and antiaging properties (Avalos-Llano et al. 2018). The oxidative properties of ETs vary depending on their chemical structures. In a study conducted by Gyamfi and Aniya (Gyamfi and Aniya 2002), it was found that ETs are better metal chelators rather than radical scavengers. Additionally, ETs can also be effective prooxidants in the absence of ascorbic acid. It is hence difficult to draw conclusion on the oxidative property of ETs since various studies have mentioned both the prooxidant and antioxidant capacity of ETs.

24.6.1 Ellagitannins in Fruits

Ellagitannins and ellagic acids are polyphenols mostly abundant in berries, namely, raspberries, strawberries, black raspberries, or pomegranates. Ellagitannins are known to possess many health benefits and provide protection against various chronic diseases, namely, cardiovascular diseases, cancers, and neurodegenerative diseases (Augustin et al. 2005; Erdman et al. 2007). The intake of polyphenol with the aim to prevent diseases is promising but not conclusive as more human clinical trials are required to reach a proper scientific evaluation (Törrönen 2009). Considering the fact that ETs are hydrolyzed to EAs which are further metabolized in the gastrointestinal tracts to form urolithins, it is thus of paramount importance to highlight that the healthy benefits are mainly attributed to those urolithin compounds (Garcia-Munoz and Vaillant 2014).

24.6.2 Pomegranates

The global increase in the consumption and production of pomegranates is associated to the health promising facet of the fruit (Türkyılmaz et al. 2013). The bright red color of pomegranate juice is attributed to the presence of the main constituents which are anthocyanins. The most prevalent tannins present in pomegranates are hydrolyzable tannins (HTs) including ETs and gallotannins in contrast to condensed tannins (CTs) which are rarely found (Gil et al. 2000; Teng et al. 2017). Numerous studies showed that ETs isolated from pomegranates possess good antimicrobial activities against a panel of microbes, namely, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Candida albicans*. It is known that the therapeutic values of pomegranates are attributed to the presence of ETs (Henning et al. 2017). For instance, a study conducted by Henning et al. (2017) on pomegranate (*Punica granatum* L.) concluded that *Akkermansia muciniphila* play an important role in the hydrolysis of ETs to EAs in the intestine and thus contribute to the health benefits of that particular fruit. Al-Zoreky (2009) evaluated the antimicrobial properties of the fruit peel of pomegranates both in vivo and in vitro. Antimicrobial screening was performed against nine strains of microbes, namely, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, MRSA (*Methicillin-resistant*

Staphylococcus aureus), *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 10536, V517, *Salmonella enteritidis* ATCC 4931, and *Yersinia enterocolitica* ATCC 23715, reporting the highest MIC value with *Salmonella enteritidis* ATCC 4931 (4 mg/mL). Furthermore, pomegranate juice was assessed for its antimicrobial property. Out of the 12 microorganisms tested, only 4 microbes including *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas* sp. showed good antimicrobial property (Türkyılmaz et al. 2013).

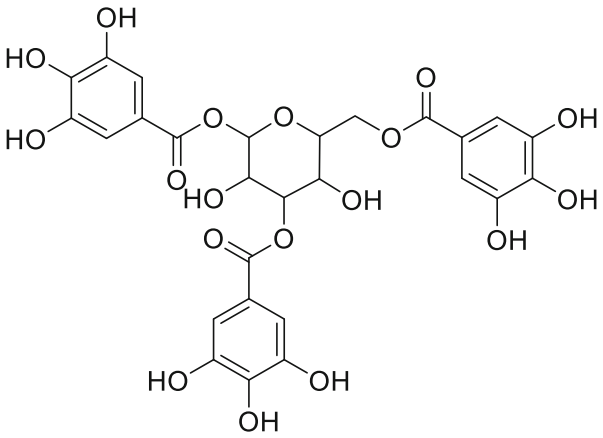
Through various studies pomegranate is found to be a potent anticancer agent that contains many bioactive components such as EA, punicic acid, and punicalagin. The extracts obtained from this plant have been found to suppress tumor cell proliferation and induce cell cycle arrest and apoptosis through modulation of various transcription factors, signaling pathways, and the expression of various genes in both in vitro and in vivo settings. Clinical studies reported pomegranate juice intake aids in the control and stabilization of prostate and colon cancer. However, very few clinical studies have been undertaken and encouraged scientists to scrutinize this promising fruit more in depth (Khwairakpam et al. 2018). Recently, for the first time, the team of Berdowska et al. (2018) evaluated the modulatory impact of selected ETs, namely, agrimoniin, sanguiin H-6, rugosins D and A, tellimagrandin I, and pedunculagin, on human breast cancer cells showing resistance to Adriamycin (MCF-7/Adr). Results showed that the highest inhibitory impact was reported with agrimoniin followed by pedunculagin, rugosins D and A, and lastly tellimagrandin I (Berdowska et al. 2018).

24.6.3 Berries

Berries are represented by a number of small fruits in a variety of colors, namely, red, blue, or purple. The most common berries are blueberry, bilberry, cranberry, blackberry, raspberry; black, white, or red currant; and strawberry, and less common include lingonberry, cloudberry, elderberry, honeyberry, whortleberry, and chokeberry. They contain high levels of polyphenols including flavonoids (anthocyanins, flavonols, flavanols), condensed tannins (proanthocyanidins), hydrolyzable tannins (ETs, gallotannins), phenolic acids (hydroxybenzoic acid, hydroxycinnamic acids, chlorogenic acid), stilbenoids, and lignans (Vendrame et al. 2016; Nile and Park 2014; Manganaris et al. 2014).

Raspberry also known as nature's candy has been consumed by the world population since thousands of years ago. They are consumed in different ways: fresh, frozen, dried and in yoghurts, desserts, cakes, juice, or jellies. Raspberries are known to be a good source of polyphenols such as anthocyanins, flavonols, and ETs which have the capacity to reduce the risk of oxidative damage caused by free radicals and have the potential to reduce the risk of chronic diseases including cancer and heart disease (Megan 2018). According to the US Department of Agriculture (USDA) National Nutrient Database, one cup of raw raspberries equivalent to 123 g contains 64 g energy, 1.48 g protein, 0.80 g fat, 14.69 g carbohydrate, 31 mg

Fig. 4 Gallotannin



calcium, 0.85 mg iron, 32.2 mg vitamin C, and 1.87 mg niacin (USDA National Nutrient Database, 2019). The two horticulturally valuable raspberries are the red (*Rubus idaeus* L.) and the black raspberries (*Rubus occidentalis* L.) (Yazdanpour et al. 2018).

The bioavailability of red raspberry anthocyanins (ATs) and ETs was studied by Ludwig et al. (2015). A total of eight ATs, two EA conjugates, two ETs, one hydroxycinnamate, and one phenolic acid were identified and quantified in the raspberry supplement using HPLC-PDA-MS. Figure 4 illustrates the chemical structures of some major compounds of interest. In the same study, volunteers were fed with red raspberries, and their plasma and urine samples were tested by UHPLC-MS. It was found that ATs and ETs undergone extensive degradation while passing through the digestive system of body to produce a variety of metabolites including methyl, glycine, glucuronide, and sulfate derivatives of phenolic acids, phenylpropanoids, ellagic acid, and urolithins. It is with respect to the produced metabolites that the red raspberries possessed the underlying protective effects on the human health. In a review compiled by Teng et al. (2017), it was suggested that future studies should be focused on the absorption of anthocyanins and how it can be used in chemotherapy and anti-inflammation treatment.

Black raspberries (BRBs), the sister berry of red raspberries, locally known as "Bokbunja" in Korea, are largely cultivated in the southern parts of Korea, China, and Japan. The cultivation area of this species has increased due to the huge demand by the population for their usage as a traditional herbal medicine. BRBs are rich in flavonoids, tannins, triterpenosides, and phenolic compounds, and these metabolites possess multiple pharmacological properties, namely, cell proliferation inhibition and apoptosis stimulation in HT-29 human colon cancer cells and anti-fatigue, anti-gastropathic, anti-inflammatory, antirheumatic, and antioxidant activities (Jeong et al. 2010). At the time of writing, due to its extraordinary functionality, different types of processed products, such as sugar extracts, wine, and vinegars, have been actively produced to create high-value added Bokbunja food products (Song et al.

2016; Kim et al. 2005). Black raspberries are heavily screened in terms of their anticancer property.

BRBs harbor a wide array of bioactive metabolites, namely, flavonols, anthocyanins, hydroxycinnamic acids, as well as ETs (Teegarden et al. 2019; Paudel et al. 2013). BRBs and other sources of dietary anthocyanins are attributed to many health-promoting benefits especially preventing eyestrain, improving night vision, and preventing macular degeneration, possess anti-inflammatory properties, and act as a protection barrier against DNA damage and anticancer and anti-neurodegenerative among others (Yousefi et al. 2015; Rezaee Kivi and Sartipnia 2013). A team of scientists in Korea studied the effects of BRBs on oxidative stress and plasma oxidant capacity in health male smokers, and the results were promising. Thirty-nine male volunteers were given 30 g of freeze-dried BR or placebo for 4 weeks. There was no age difference, neither smoking history or anthropometry nor nutrient intake between groups. It was found that the BRB supplementation had no effect on plasma lipid profiles, LDL oxidation, and DNA damage. However, the activity of the antioxidant enzymes increased drastically together with glutathione peroxidase and catalase and reduced plasma lipid peroxidation. Thus it was concluded that BR supplementation might decrease cigarette smoke-induced oxidative stress through increase of endogenous antioxidant enzyme activities (Park et al. 2015).

The seeds of BRBs are known to contain a large quantity of ETs. As mentioned earlier, ETs are hydrolyzed by the system of the body to subsequently form ellagic acids (EAs) which are further metabolized into urolithin A (UA) and urolithin B (UB) known to be bioavailable in the colon and prostate. Cho et al. (2015) conducted an interesting study on those compounds (ETs, EAs, UA, UB) in relation to their anticancer activities using HT-29 colon cancer cells. Results showed that EA caused a slight but significant cell cycle arrest at the G1 phase, and both urolithins caused cell cycle arrest at the G2/M phase and upregulated p21 expression. Apoptotic cells were detected by annexin V-FITC/PI assay when treated with the compounds. ET, EA, UA, and UB showed anticancer activity by arresting the cell cycle and inducing apoptosis on the HT-29 human colon cancer cells. Therefore, this study suggested that the BRB seeds could be a potential source of anticancer ETs.

Interestingly, BRBs exhibited good antioxidant properties due to the presence of anthocyanins and phenolic acids. The ethanolic extract (70%) containing compounds 9, 11, 16, and 17 has the highest antioxidant potential with IC50 <0.1 μg/mL, while the ethanolic extract (30%) containing compounds 8, 9, 11, and 17 showed an activity with IC50 value 2.80 μg/mL (Xiao et al. 2017). A study was conducted on the level of polyphenolic contents with respect to the maturation stages of Java plum (*Syzygium cumini* Lam.). Data showed that the level of gallotannins, ellagitannins, flavonols, gallic acid, and ellagic acid decreased as the fruit ripened (Lestario et al. 2017).

Strawberry (*Fragaria x ananassa* Duch.) is one of the most popular and appreciated berries consumed by most people either fresh or dried or processed. It is reported that strawberries help to prevent inflammation; fight against oxidative stress and obesity; reduce risk of heart disease, diabetes, and neurodegenerative disorders;

and also act as a preventive barrier against different types of cancers (Afrin et al. 2016). According to previous studies, the ET contents of raspberry and strawberry were 160 and 25–59 mg/100 g in fresh samples, respectively. However, Koponen et al. (2007) reported a lower content value ranging from 2 to 66 mg/100 g. EA is the main phenolic acid in strawberries (from 300 mg to 600 mg/100 g) (Vendrame et al. 2016). ETs and EAs are reported to play a primordial role in controlling hyperglycemia and hypertension in diabetic patients. Recently, a study conducted by Nowicka et al. (2019) demonstrated that ETs and procyanidins play an important role in terms of antioxidant properties of strawberries. Strawberry has good anti-inflammatory property, and the compound pelargonidin-3-*O*-glucoside (P3G) is the major anthocyanin responsible for this property (Duarte et al. 2018). Fumagalli et al. (2016) investigated anti-inflammatory activity after simulated gastric digestion conducted in vitro. It was concluded that strawberry tannins exerted the same anti-inflammatory property even after digestion. To the best of our knowledge, very few studies have been conducted on strawberries in relation to the compound ET and thus require more investigation.

Blueberry (*Vaccinium corymbosum* L.) is among the most commonly consumed berries in the United States. Along with blackberries and strawberries, blueberries (BBs) are rich sources of ETs (up to 600 mg/100 g), followed by chokeberries, cloudberries, and red raspberries (approximately 260 mg/100 g) (Zanotti et al. 2015). Of much interest, it is important to highlight that from a study conducted by Seeram et al. (Bianca Fuhrman 2006), the phytochemical profiling of BBs revealed that the predominant types of tannins present in them are condensed tannins (proanthocyanidins) rather than hydrolyzable tannins (namely ETs) which are the most abundant compounds in strawberries, blackberries, and raspberries. Johnson et al. (2015) conducted various studies on 480 mL blueberry juice which is equivalent to a cup of fresh BBs. Results showed that these fruits have the potential to reduce the systolic and diastolic blood pressure and increase superoxide dismutase activity after the consumption of BBs. It was thus concluded that daily consumption of BBs can potentially reduce blood pressure and arterial stiffness. Basu et al. (2010) also confirmed the health benefits of BBs in terms of cardiovascular diseases and metabolic syndrome. Johnson et al. (Johnson and Arjmandi 2013) reviewed the anticancer property of BBs and mentioned that these berries showed promising anticancer effects by preventing carcinogenesis and DNA damage. However, further clinical trials are required with respect to their cancer-preventive ability.

24.6.4 Ellagitannins in Vegetables

Vegetables are of minor importance to polyphenol intakes. Carotenoids are obtained mainly from vegetables and sterols from margarines, bread, and fruit. Isoflavonoids came from soy products, and lignans were derived from seeds, soy

products, rye bread, and other cereal products (Ovaskainen et al. 2008). Koponen et al. (2007) screened various vegetables, namely, eggplant, lettuce, red paprika, potato, red beet, red cabbage, red onion, red radish, rhubarb, and tomato, for the total content of anthocyanins and ETs. Results showed the content that total anthocyanins ranged from 3 to 75 mg/100 g of fresh weight and the total ellagic acid content varied from 1 to 330 mg/100 g of fresh weight. As far as our literature search could establish, there are very few articles reading ETs present in vegetables. Most of the research articles revolve around pharmacological properties of ETs present in berries. As a consequence more scientific investigation should be carried out in order to better clarify on the pharmacological properties and health benefits of ETs in vegetables.

24.6.5 Ellagitannins in Nuts

Nuts are considered as highly nutritious foods in many parts of the world and are easily found in many dishes and even salads (Sanchez-Gonzalez et al. 2017). Common edible tree nuts are almonds, Brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut (Chang et al. 2016). Nuts are nutrient-rich foods and have been a regular constituent of mankind's diet. Nuts are convenient and tasty snacks that contribute to a healthy dietary pattern. They are usually consumed as whole nuts (as raw, roasted, or salted) (Alasalvar and Shahidi 2008). It is known that frequent nut intake is associated with protective effects against cardiovascular diseases. In addition to the generally high contents of unsaturated fatty acids and polyphenol, compounds seem to be also implicated in health-promoting effects of nuts due to their antioxidant properties (Abe et al. 2010).

Le et al. (2014) conducted a study on cytotoxic effects of ETs derived from walnuts. The targeted human cancer cells were MDA-MB-231, MCF7, and HeLa cells. Interestingly, the methanolic extract revealed good cytotoxic activity against all of the cancer cells tested and suggested that this effect was attributed to the presence of compounds isolated from the extract. The compounds were identified as tellimagrandin I and tellimagrandin II considered as members of the ET family (Fig. 5). Anthocyanins and ellagitannins derived from nuts and berries were considered to be potent cardiometabolic biomarkers as they significantly reduced the cholesterol level compared to pomegranates and grapes (Garcia-Conesa et al. 2018).

US pecans and Chinese hickory nuts are known to possess a panel of different phytochemicals including phenolic acids and proanthocyanidins attributed to health promising benefits to human beings. Gong and Pegg (2017) conducted a study on the isolation of ellagitannin-rich phenolics from those nuts and found that US pecans possess more proanthocyanidins in contrast to Chinese hickory nuts. Comandini et al. (2014) analyzed the complete profile of tannins along with other phenolic compounds present in commercial chestnut bark samples using HPLC-DAD/ESI-

MS method. A total of seven compounds, namely, vescalin, castalin, gallic acid, vescalagin, 1-*O*-galloyl castalagin, castalagin, and EA, were isolated, and the compound, 1-*O*-galloyl castalagin, was tentatively identified and found for the first time in chestnut bark samples (Fig. 6).

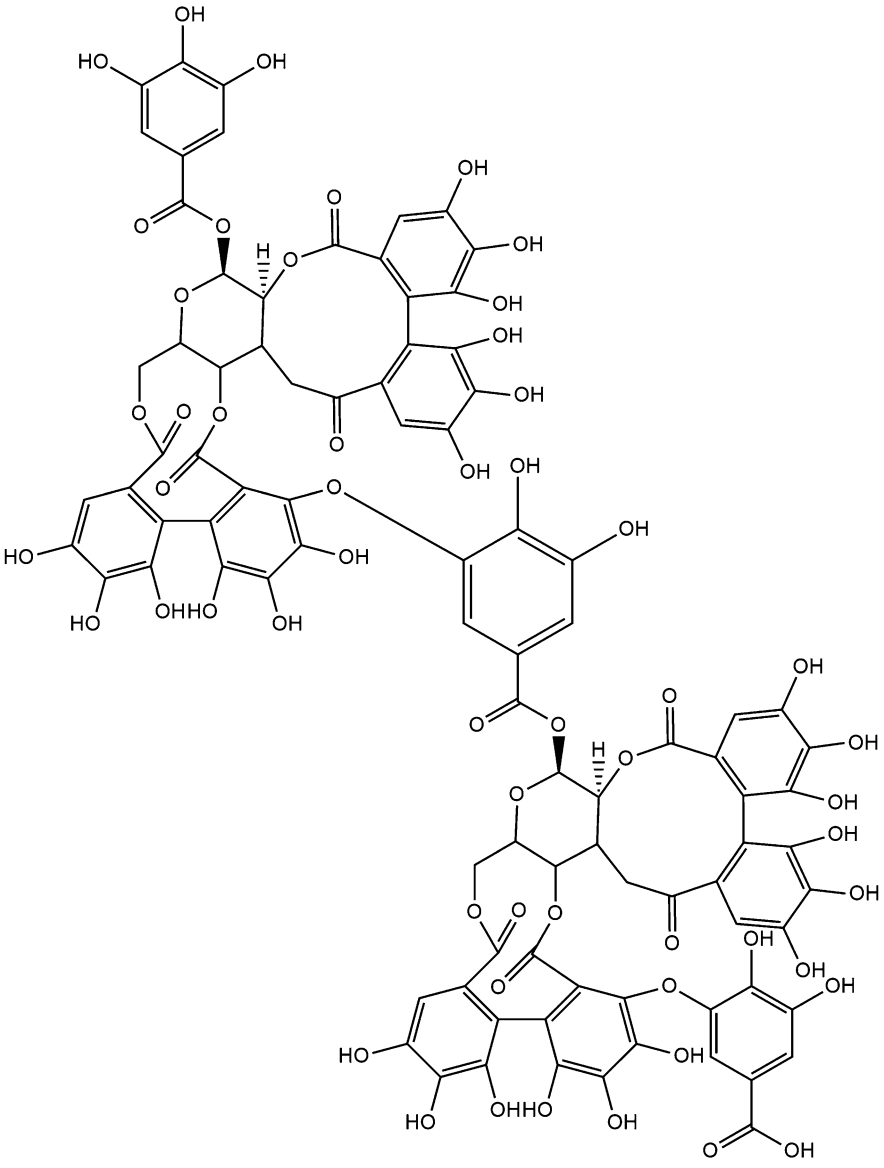
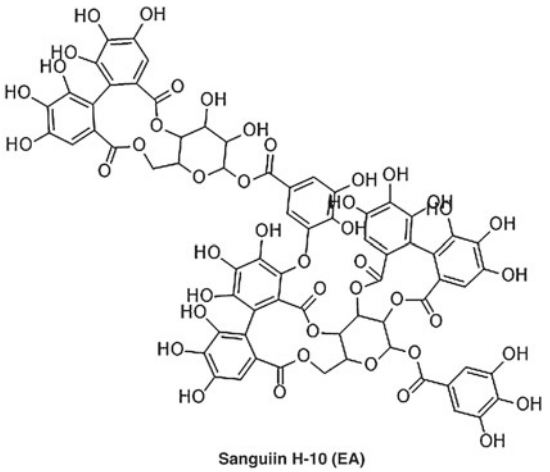
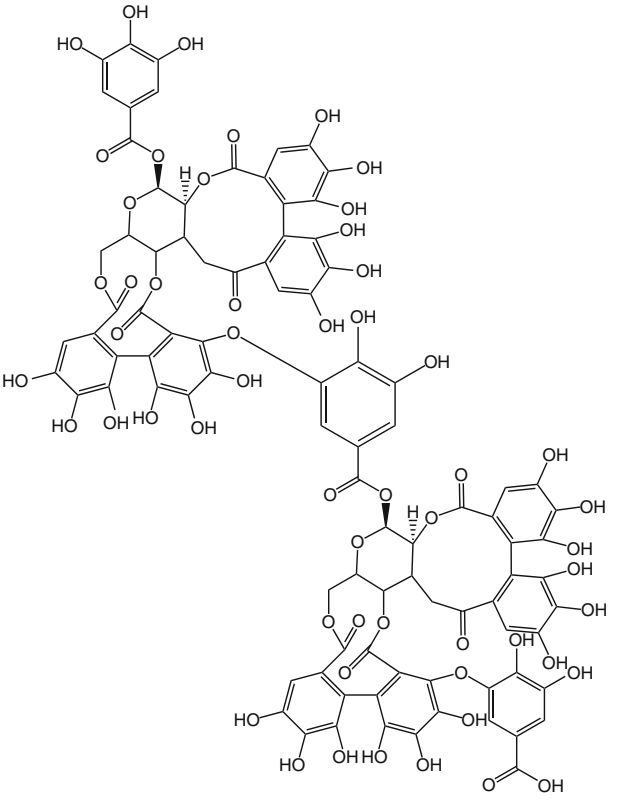


Fig. 5 (continued)



Sanguiin H-10 (EA)



Lambertianin C (EA)

Fig. 5 Anthocyanins (ATs), ellagitannins (ETs), and ellagic acids (EAs) isolated from red raspberries

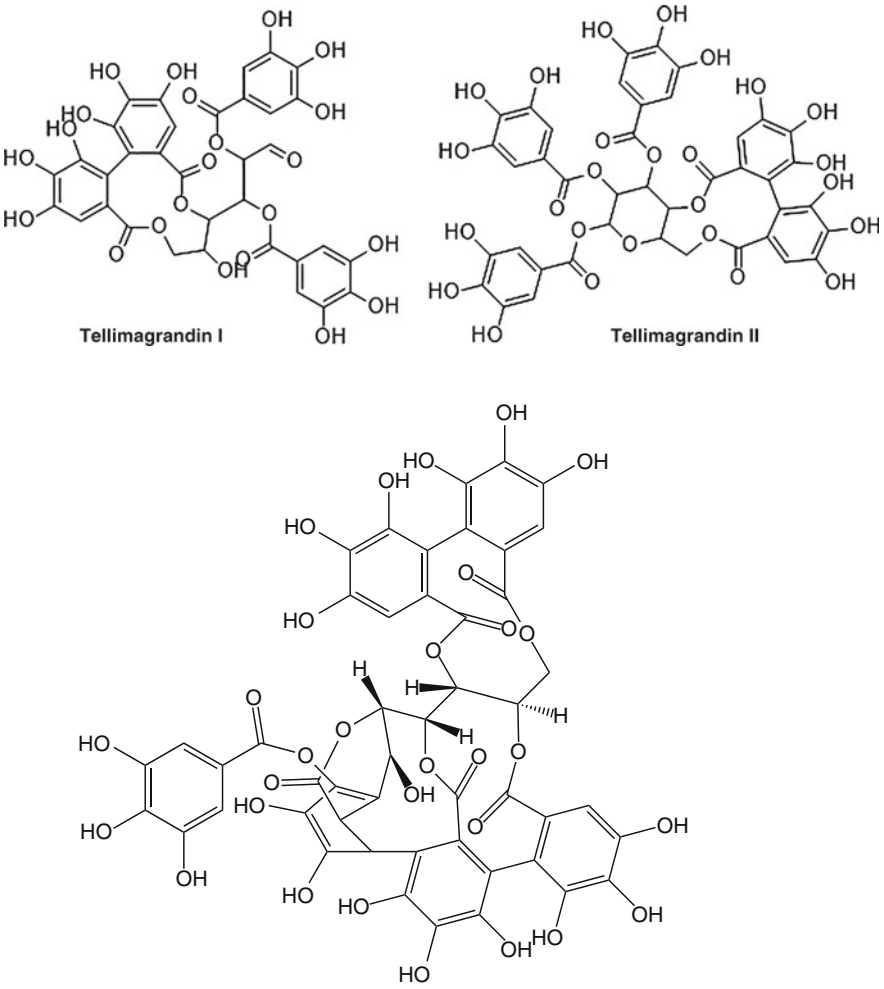


Fig. 6 (**a**) Tellimagrandin I, (**b**) tellimagrandin II, (**c**) 1-*O*-galloyl castalagin

24.7 Application in Foods

ETs have attracted the interest of numerous researchers because of their recently recognized role as promising chemotherapeutic agents. There are also recent studies on their occurrence in fruit–vegetable and processed products. Berries are very popular among fruits. They are widely consumed in fresh forms and as food products such as preserves, jams, yoghurts, and ice creams. In China, longans (*Dimocarpus longan*) are consumed as fresh, dried, or canned in syrup. They are equally used in liqueurs or drinks. The seeds of longans possess therapeutic values since they contain tannins in the form of galloyl-HHDP-glucopyranose and pentagalloyl-

HHDP- glucopyranose. Fruit juices are popular products, and a large variety of new products based on fruit juices have recently appeared in the market. In particular, pomegranate and berries juices are mixed with other juices because of their intense flavor and color. These fruit juices are a very rich source of ETs and other phenolic compounds. ETs are important for fruit juices due to their strong antioxidant activity. On the other hand, these compounds cause serious defect such as haze and sediment formation in fruit juice (Bakkalbasi et al. 2009).

The health benefits offered by probiotic fermented milks can be increased by adding native fruit pulps. Red fruits containing EAs and ETs exhibit powerful antioxidant properties and other beneficial biological properties, namely, cardioprotective effects, prevention of human pathogenic bacteria, α-amylase inhibition of angiotensin I-converting enzyme, and proliferative activities against various cancer cells. A study conducted by Bueno and team aimed at improving the physical properties of symbiotic yoghurts through their supplementation with a mixture of red berry pulps: strawberry, raspberry, and "pitanga." The simplex-centroid design was used for mixture modelling. The design included seven batches/trials: three supplemented with each type of fruit pulp, three corresponding to the binary mixtures, and one supplemented with the combination of the three pulps. A control experiment was prepared without addition of fruit pulp. Processed milk bases were fermented at 42 °C until pH 4.7 by using a starter culture blend that consisted of *Streptococcus thermophilus* (TA040), *Lactobacillus bulgaricus* (LB340), and *Lactobacillus acidophilus* (LA140). Subsequently, milk base (80%) was blended with a pulp preparation (20%) and other ingredients according to the experimental design. Physicochemical analyses, enumeration of viable bacteria, and color and rheological characteristics of the yoghurts were evaluated 36 h after preparation and 21 days after cold storage. Three regression models were adjusted to the results (linear, quadratic, and cubic special). The results showed that the addition of a mixture of red fruit pulps affected pH and counts of probiotic bacteria, which slightly decrease during storage. Addition of the three red berry pulps simultaneously enhanced the color and rheological properties of probiotic yoghurts when compared to the control. It was possible to estimate the optimum mixture compositions of the three fruit pulps that increase EA contents in probiotic yoghurts (Luciana et al. 2014).

Trigueros et al. (2014) conducted a study on the antioxidant activity and protein–polyphenol interactions in a pomegranate (*Punica granatum* L.) yoghurt. Pomegranate juice (PGJ) is rich antioxidants; however phenolics easily interact with proteins. A yoghurt fortified with 40% PGJ made from arils was studied (PGY) to determine the antioxidant activity and to estimate the phenolics–proteins interaction during 28 days of cold storage. Juice, yoghurts, and protein-free permeates were analyzed for phenolic composition. It was observed that the anthocyanin profile was changed by the fermentation of the yoghurt, especially the content in cyanidin-3-*O*-glucoside. However, during storage, the anthocyanin content in the fortified yoghurt decreased, but no color change in the yoghurt was noted. The analysis of permeates revealed that the degree of phenol–protein interaction depends on the type of phenolic, EA, and delphinidin 3,5-*O*-diglucoside being the least bound phenolic compounds. The presence of PGJ in yoghurt enhanced radical scavenging performance, whereas all the observed ferric reducing power ability

of PGY was attributed to the presence of PGJ. The 84.73% of total anthocyanins remained bound to proteins at the first day of storage and 90.06% after 28 days of cold storage, revealing the high affinity of anthocyanins for milk proteins.

Another study by El-Said and team on the antioxidant activities and physical properties of stirred yoghurt fortified with pomegranate peel extract (PPE) was conducted. Stirred yoghurt was prepared from reconstituted skim milk powder fortified with 5%, 10%, 15%, 20%, 25%, 30%, and 35% of the pomegranate peel extract (PPE). Results showed that yoghurt fortified with PPE demonstrated higher antioxidant activities compared to the negative controls. Addition of PPE had no significant effects on the sensory attributes (appearance, body and texture, or flavor) as compared to the control sample. Increasing the percentage of the added PPE resulted in decrease in the viscosity of the stirred yoghurt, but samples containing 20% and 25% PPE led almost the same viscosity (El-Said et al. 2014).

Ice cream is one of the most consumed dairy products in the world. Ice cream is rich in macronutrients, i.e., carbohydrates, fats, and proteins, and some micronutrients, i.e., vitamins A, D, and E and calcium mineral. However, commercially available ice creams are generally poor in natural antioxidants like vitamin C, colors, and phenolics. Addition of pomegranate peel phenolics at 0.5 and 1.0% (w/w) showed significant improvement of the antioxidant and α-glucosidase inhibitory activities of the enriched ice creams compared with control sample. Antioxidant activity as EC_{50} and α-glucosidase inhibitory as IC_{50} of 1.0% phenolic-enriched ice creams were 133.3 and 22.9 μg/mL, respectively. More than 75% of the panelists accepted the phenolic-enriched ice creams in sensory evaluation, which lends supports to such products for commercial introduction to the general public with the potential as functional foods (Çam et al. 2014). Sagdic et al. (2012) studied the interaction between some phenolic compounds and probiotic bacterium in functional ice cream production. The radical scavenging activity supplemented with EA, gallic acid (GA), PPE, and grape seed extract (GSE) was higher compared to the control. It was observed that the highest radical activity was attributed to PPE due to its high phenolic content. The study concluded that EA, GA, PPE, and GSE could be used to enhance the phenolic content of ice cream and to gain antioxidant property.

Kushwaha et al. (2015) suggested an economical approach for the production of purified ETs powder from fresh and fermented peels of pomegranate fruit. The method involved the inoculation of about 500 g of fresh pomegranate peel with *Aspergillus niger* culture (3. 8 × 109 spore per ml/100 g fresh peel) and incubated at 26 °C for 7 days in an incubator. In five conical flasks of 500 mL, about 100 g of fermented pomegranate peel was added to 200 mL distilled water and shaken for 18 min at 90 °C. Each flask was centrifuged and the supernatant was collected. The supernatant was subjected to different analysis, namely, antioxidant activity and ET content. The antioxidant activity of ETs powder from fermented peel was slightly lower than ETs powder from fresh peel. It may be due to the oxidation degradation of phenolic compounds during fermentation. After purification of ETs extract from fresh and fermented peel, content of TEC and respective antioxidant activity increased in ETs powder. So, ETs powder may be considered as an ultimate product for its further application, e.g., as biopreservatives, nutraceutical, additives, and antioxidative agent in different food, pharma, and cosmetics products. The most economical approach

proposed was from 1 kg fresh peel; approximately 37–38 g of purified ETs powder can be produced of worth Indian rupees 25,441 in the international market.

24.8 Safety: Toxicity and Side Effects

Oak leaves have a high concentration of ETs. These phytochemicals can either be beneficial or poisonous to animals. For instance, beef cattle are often intoxicated after consuming oak leaves. The severity of the poisoning has recently been associated with the ruminal microbiota, as different bacterial populations were found in animals that tolerated oak leaves and in those that showed clinical and pathological signs of toxicity. Intoxication has previously been linked to the production of phenolic metabolites, particularly catechol, phloroglucinol, and resorcinol. It was projected that the microbial metabolism of ETs could be linked to the different tolerance level or intoxication in different animals. Consequently, it is important to understand the metabolism of ETs in cattle. Different urolithins were detected in ruminal fluid, feces, urine, and plasma. Oak leaf ETs were noticed to decrease as they were converted to urolithins, namely, isourolithin A and urolithin B, by the ruminal and fecal microbiota. Urolithin aglycons were observed in rumen and feces, while glucuronide and sulfate derivatives were detected in plasma and urine. Sulfate derivatives were the main metabolites detected in plasma, while glucuronide derivatives were the main ones in urine. The main urolithins produced in cattle were isourolithin A and urolithin B. Low molecular weight phenolics of the benzoic, phenylacetic, and phenylpropionic groups and metabolites such as catechol, resorcinol, and related compounds were also detected. There was a large variability in the kinetics of production of these metabolites in individual animals, although they produced similar metabolites in all cases. Further studies are needed to demonstrate if the efficiency in the metabolism of ETs by the microbiota could explain the differences observed in susceptibility to intoxication by the different animals (González-Barrio et al. 2012).

24.9 Marketed Products

No marketed product reported

24.10 Patents

No patents reported

24.11 Conclusion and Perspectives

The pharmacological aspects, health benefits, chemistry behind ETs, application of ETs in foods, and toxicity discussed in this chapter, are yet another example of the remarkable impact of natural products on human activities. It is very fascinating to

realize that natural products derived from the metabolism of plants for their protection against pathogens and herbivores, then identified as active principles of plant extracts used in folk medicines and later as promising drug leads, end up being investigated for their role in the elaboration of the biophysicochemical and organoleptic profile of a beverage particularly wine. The biological activity and physiological effects of ETs and EA have to be consistent with their bioavailability and metabolism. Thus, results obtained from in vitro studies using cultured cells that would not be in direct contact in vivo with food ETs should be considered with caution. This is, for example, the case of studies testing activity of ETs on liver or breast cancer cell lines. The metabolic transformation of ETs into urolithins A and B is a widespread phenomenon in nature. According to the present knowledge, berries of *Rubus*, *Fragaria*, and *Rosa* species, as well as pomegranate and muscadine grape products and walnuts, are the best dietary sources of ellagitannins. Due to limited food sources, the dietary intake of ETs and EA is probably low compared to many other polyphenols. Experimental studies on ellagitannin-rich foods have demonstrated potential for health benefits, and dietary interventions are being conducted in patients at increased risk for cancer development. In addition to high levels of ETs, berries, pomegranates, and walnuts also contain a variety of other phytochemicals, essential fatty acids, and other nutrients and may therefore be regarded as "health foods" or even "superfoods." Research is progressing in many laboratories and will eventually provide new evidence on the health effects of dietary ETs. Meanwhile, increased consumption of the delicious ET-rich foods and beverages as a part of a balanced diet can be recommended. For example, pomegranate fruit (*P. granatum* L.) yields the richest source of ETs among other fruit juices and is recommended to consume frequently. The ellagitannins found in pomegranate fruit are very potent antioxidants, and pomegranate juice exceeds the in vitro antioxidant potency of other common commercial fruit juices. Pomegranate peel is a rich source of phenolics which contain unique phenolic compounds called punicalagins in significant amounts. Utilization of pomegranate by-product as a source of functional ingredient in food industry is an ideal objective. This study clearly showed that phenolics of pomegranate peels in microencapsulated form would be a suitable ingredient for improving the functional properties, precisely antioxidant and α-glucosidase inhibitory activities of ice cream. Future studies are necessary to investigate the interaction of phenolics with other food components in ice cream.

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Gallotannins in Food

25

Ipek Suntar, Fabiana Labanca, and Luigi Milella

Contents

Abstract

Tannins are natural polyphenolic components found in fruits such as grapes, apples, pears, plums, peaches, strawberries, and cranberries as well as in beverages including wine and tea. Other sources of tannins are *Acer ginnala* Maxim., *Caesalpinia spinosa* (Molina) Kuntze, *Caesalpinia brevifolia* Baill., *Hamamelis virginiana* L., *Quercus infectoria* Oliv., *Terminalia chebula* Retz., *Eucalyptus sieberiana* F. Muell., and *Schinopsis* Engl. species. Tannins can be classified into hydrolyzable and nonhydrolyzable (condensed) tannins. Hydrolyzable tannins possess a polyhydric alcohol group in center and hydroxyl groups that are esterified by gallic acid or hexahydroxydiphenic acid,

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called gallotannins and ellagitannins, respectively. Medicinal plants containing gallotannins, *Rhus chinensis* Mill. and *Terminalia chebula* Retz., have been prescribed for the treatment of cough, constipation, dysentery, and dysfunctions of the liver and kidney in traditional Chinese medicine. Gallotannins, belong to antioxidant class of polyphenols, seems to be involved in a wide variety of mechanisms linked to human health. Despite their important biological activities, ingestion of large quantities of these compounds may cause some adverse effects. The number of clinical studies on gallotannin containing natural sources is not adequate; therefore, further studies on the potential adverse effects that might be associated with high gallotannin consumption are needed. Consequently, it can be concluded as tannins in optimal doses have promising effects for human health; however, high amount of tannins is not recommended due to its possible risk in cancer induction, anti-nutritional effects, and other adverse reactions.

Keywords

Tannin · Gallotannin · Tannic acid · Bioactivity · Safety

Abbreviations

PGG β-1,2,3,4,6-pentagalloyl-*O*-D-glucopyranose

25.1 Introduction

Tannins are the most complex group of polyphenols. They comprise condensed tannins (or proanthocyanidins), phlorotannins, and hydrolysable tannins. This last class includes soluble chemical compounds of molecular weight comprised between 500 and 5000 Da and characterized by the presence of a core polyol, generally D-glucose. Among them, gallotannins and ellagitannins are the most representative. Their distinctive feature, important in food quality, is the astringency, a sense of dryness extended to all mouth, due to the hydrogen bonds formation between phenolic hydroxyls and salivary proteins responsible of their precipitation as complexes (Arapitsas 2012). The case of tannins is the most representative example of secondary metabolites role in plants. On one side, their astringency and bitterness ensure the defense against herbivores; on the other, their effectiveness in human health makes them recognized beneficial compounds and allows their large use in natural medicine. Although the aim of this book is not the analysis of phytochemicals in medicinal plants but, instead, in food plants, it is interesting to note what large use of these compounds has been done in the past in the care of many diseases. Traditional Chinese medicine prescribes several medicinal plants, containing gallotannins, to treat a large variety of conditions, as well as the galls of *Rhus chinensis* Mill. to dysentery and the fruits of *Terminalia chebula* Retz. that represent the main components of Ayurvedic remedy "Triphala," to care liver and kidney dysfunctions. They are also used in dry form for cough and constipation (Tewari et al. 2017). The fruits of *Cornus officinalis* Siebold & Zucc. are useful in erectile dysfunction (Sieniawska and Baj 2016). *Paeonia suffruticosa* Andrews is

Fig. 1 Main traditional uses of gallotannin-rich plants

highly revered in Chinese culture, and its root bark is used to improve liver function and to reduce fever, just like *Sanguisorba officinalis* L., known as Di Yu, that presents the same qualities and, moreover, shows antihemorrhagic and wound curative properties (Bensky et al. (1993) Chinese Herbal Materia Medica. Eastland pr). Gallotannins in *Hamamelis virginiana* L. have been used for the first time by Indians in North America, for their anti-inflammatory and astringent properties and to care hemorrhoids and diarrhea (Okuda and Ito 2011), while *Caesalpinia spinosa* (Molina) Kuntze pod infusion is used as gargle for inflamed tonsils. A summary of mostly recognized medicinal properties of gallotannin-rich plants is reported in Fig. 1. The application of gallotannins could be extended also to other fields, i.e., the use of tara gum in food industry for the emulsification and stabilization properties (Wu et al. 2015) or in chemical industry as antifouling against undesirable accumulation of marine organisms on boat hulls (Bellotti et al. 2012). In textile sector, the treatment of cotton with oak gall extract provides antibacterial tissues, useful in Candida infections' reduction (Tayel et al. 2013). Furthermore, gallic acid (GA), deriving from gallotannins metabolism, shows an important pharmaceutical application in the production of trimethoprim, an antibacterial drug used in association with sulfonamide in the treatment of various infections (Sarıözlü and Kıvanç 2009).

25.2 Bioactive Constituents

Gallotannins originate from the esterification of the hydroxyls of the sugar core with gallic acid (3,4,5-trihydroxyl benzoic acid) that was discovered for the first time in the middle 1700s by a German chemist, Carl Wilhelm Scheele (Arapitsas 2012). The biosynthetic origin of gallic acid was disputed for long time between an early

derivate of shikimic acid and phenylalanine, a later product of shikimate pathway (Fig. 2). Dewick and Haslam (1968, 1969) through radioisotope tracer experiments proved the maintenance of carboxylic group of shikimic acid in gallic acid, confirming the first theory. In the formation of gallotannins, the esterification of glucose with gallic acid by glucosyltransferase provides the first intermediate, the glucogallin (1-galloyl-β-glucose) that has a pivotal role in subsequent esterifications as acyl donor (Daglia et al. 2014) (Fig. 3). The simplest gallotannin is pentagalloyl glucose (β-1,2,3,4,6-pentagalloyl-*O*-D-glucopyranose) or PGG that presents five ester linkages between sugar hydroxyl groups and carboxylic groups of gallic acid. More complex gallotannins are derived from galloylation of this unit to yield hexa-, hepta-, octa-, and galloderivatives. The enzymatic acylation of pentagalloylglucose

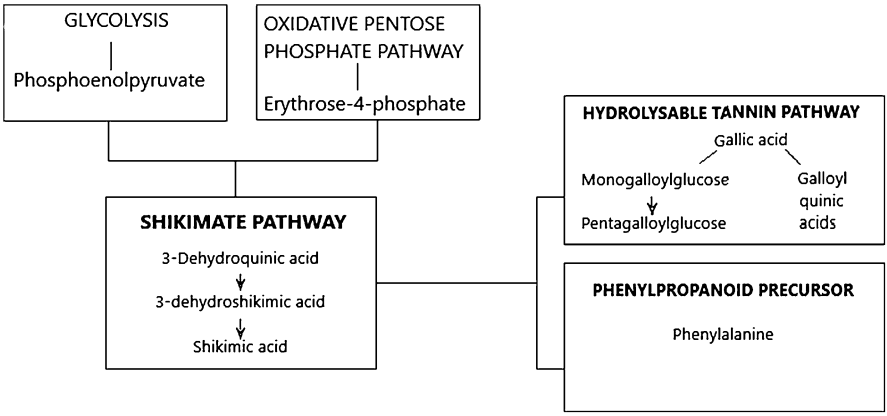

Fig. 2 Shikimate pathway. (Romani A, Lattanzio V, Quideau S (2014). John Wiley & Sons)

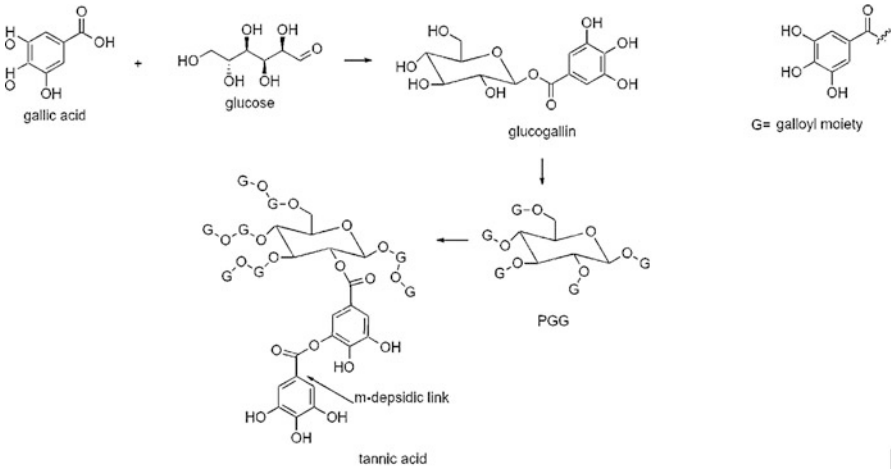

Fig. 3 Proposed biosynthetic pathway for gallotannins

was demonstrated in vitro. In particular, four galloyltransferases have been identified in sumac leaves (Zhang et al. 2009). Further esterification with another five molecules of gallic acid results in the most known gallotannin, the tannic acid. In the eighteenth century, studies on tannic acid led Adolph Strecker to establish that it could be hydrolyzed in gallic acid and glucose and to provide its first formula $C_{27}H_{22}O_{17}$ (Arapitsas 2012). Today it has been revised in $C_{76}H_{52}O_{46}$. Thus, its chemical structure should be represented by that of a decagalloyl glucose, but instead in nature it is variable, depending by species, ranging from 2 to 12 galloyl residues per molecule (Sieniawska and Baj 2016). It is a largely used food flavoring, according to European Union regulation (EC) No. 2232/96 (Arapitsas 2012). Its antioxidant activity revealed stronger than that of vitamin E by DPPH and ABTS radical assays, making it an appreciated preservative in food (Badhani et al. 2015).

Polygalloyl esters were firstly identified more than 100 years ago by Fischer, together with Freudenberg. They formation proceeds by *meta*- or *para*-depside bonds that involve galloyl units' addition to phenolic hydroxyls in *meta* or *para* position with respect to galloyl carboxyl group. It must be highlighted that the nature of sugar hydroxyls is different from that of phenolic hydroxyls. For this reason, they are more simply hydrolysable than ester bonds, and it is easy to isolate PGG from a mixture of polygalloyl esters. The degree of polymerization and galloylation, as reported below, is important because it affects the chemopreventive properties of gallotannin-rich extracts. The presence of several chiral centers in all gallotannins is responsible of the existence of many isomers, characterized by the same molecular weight, but by different susceptibility to hydrolysis and different elution times during chromatography. Also their ability to precipitate proteins is related to structure. The occurrence of numerous structures and their variability is due to oxidative coupling of other gallic acid molecules or to phenolic oxidation. As told above, it has been shown that the shikimic acid plays a central role in the biosynthesis of gallotannins. Even from its hydration, it is possible to obtain the quinic acid that combines with gallic acid to provide a new class of gallotannins, the galloquinic acids (Fig. 2) (Karas et al. 2017). In these molecules, the hydroxyls of quinic acid are esterified with gallic acid to provide differently substituted galloquinic acids. The major limit of gallotannins and, in general, of hydrolysable tannins isolation and quantification is represented by the absence of commercially available standards and specific detectors that lead to the use of nonselective methods to isolate them, with less accuracy (Arapitsas 2012). Another consideration derives from the high susceptibility to hydrolysis of *meta*-depside gallotannins that makes them not extractable by acid methods or with high temperatures (i.e., Soxhlet extraction), because they are promptly hydrolyzed in PGG (Barreto et al. 2008). The classic method for hydrolysable tannins extraction is methanolysis that causes the release of methyl gallate that could be overestimated (Gan et al. 2018). However, given the importance of gallotannins for human health, their occurrence among plants and their extraction have been studied since ever, as reported by Okuda et al. (1993) who investigated on the presence of hydrolysable tannins in herbaceous and woody plants, in the attempt to find a correlation with plant evolution. In more recent studies, they correlated progressive oxidation of compounds with morphological

evolution (Okuda et al. 2000) and proved that, in herbaceous plants, gallotannins structures remain invariant, but in woody plants, they transform in the early part of season, sometimes even changing from gallotannins to ellagitannins, as in *Liquidambar formosana* Hance (Okuda and Ito 2011). Indeed, not all compounds are stable and isolable. Among all gallotannins that have been isolated, the food interesting compounds are numerous.

- *Hamamelitannin.* This digalloylhamamelose has been originally isolated from a *Hamamelis* species, in particular from *Hamamelis virginiana* L. (Hamamelidaceae). Its galloyl esters are present also in *Castanea* species (Fagaceae) and *Sanguisorba* species (Rosaceae), the first consumed cooked as autumnal fruit, or in jam preparations, the second eaten raw or cooked in salads or soups (Okuda and Ito 2011).
- *3,4-; 4,11-; 3,4,11-tri-O-Galloylbergenin*. This C-glycosidic gallotannin is present in *Mallotus japonicus* (L.f.) Müll.Arg. (Euphorbiaceae). This plant is often called "The food wrapper plant" because of the large leaves that are used to wrap food in Japan. They are edible once they are cooked (Saijo et al. 1990).
- *Penta-O-galloyl-glucoside* and its analogs (*hexa-* to *nona-* gallosydes) (Luo et al. 2014) and *methyl gallate* (Barreto et al. 2008) in *Mangifera indica* L., commonly known as mango. Although these active compounds have been detected in major amounts in peel and kernel, their presence has been revealed in traces (<0.2%) also in the pulp (Kiss and Piwowarski 2016).
- *1,2,3-tri-O-Galloyl-β-D-glucose, geponin, penta-O-galloyl-β-glucose, and 2,6-di-O-galloyl-β-glucose* from *Geum japonicum* L. (Rosaceae). Tea of leaves and flower is used for astringent properties in order to treat cough and hemorrhages (Yang et al. 2011).
- *3-Galloylgallic and 4-galloylgallic acid, tri-, tetra-, penta-, hepta-, and nona-galloylglucose* from *Rhus chinensis* Mill. (Anacardiaceae). The seeds can be eaten for stomachache, mushroom poisoning, and indigestion; the buds boiled are useful against diarrhea, as well as fruit decoction, while dried fruits also in case of constipation, vomit, and indigestion. Fruit powder is used as antitoxin (CRC World Dictionary of Medicinal and Poisonous Plants (2012) Umberto Quattrocchi. CRC Press).
- *1,2,4-tri-O-Galloyl-β-glucopyranose and 1,3,4-tri-O-galloyl-β-glucopyranose* (Sahar et al. 1997)*, PGG, and β-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose* (Corilagin) (Xiao et al. 2013) from seed and leaves of *Punica granatum* L., better known as pomegranate.
- *Turkish gallotannins (penta-, hexa-, and hepta-galloylglucose)* from *Quercus infectoria* Oliv. (oak tree). The galls are used, in addition to other cereals, to make bread (Stashia 2013).
- *Hexa- and hepta-galloylglucose* from *Rhus typhina* L. (sumac). The fruits, big red cones consisting of individual drupes, are the ingredients of a lemon tasting drink, the "sumac-ade," used in the Middle East Mediterranean area to care gastrointestinal diseases (Kossah et al. 2011).

- *1,2,6-tri-O-Galloyl-glucose* and *1,2,3,6-tetra-O-galloyl-glucose* in *Ceratonia siliqua* L. pods, characterized by a sweet flavor. It is appreciated for gelling and thickening properties of flour deriving from seeds and for the low glycemic index that allows using it in diabetic food manufacture (Kiss and Piwowarski 2016).
- *Monogalloyl to hexagalloyl hexosides* in *Canavalia gladiate* (Fabaceae) red beans. This peculiar coat color is attributable to these compounds. Although this type of legume is the richest in antioxidant, especially in gallotannins, among other 42 Chinese edible beans evaluated, it is rarely used. The tender pods could be consumed as vegetable as a great natural source of gallotannins, but this plant is not commercially cultivated (Gan et al. 2018).
- *Gallic acid, methyl gallate,* and *ethyl gallate* in *Toona sinensis* (Juss.) M. Roem. (Meliaceae). The leaves are edible and are often employed in Chinese vegetarian cuisine (Hseu et al. 2008).
- *Gallic acid, 5-O-galloyl, 3,5-O-digalloyl,* and *3,4,5-O-trigalloyl quinic acid derivatives* are the main gallotannins in edible oil from *Pistacia lentiscus* L. (Anacardiaceae) (Romani et al. 2002).
- Galloylquinic acid, galloylshiquimic acid, galloylhexoside acid, digalloylquinic shikimic acid, digalloylquinic acid, and other mono-, di-, and tri-derivatives have been founded in different amounts in fruits and leaves of strawberry (*Arbutus unedo* L.) of various areas of Mediterranean (Miguel et al. 2014). These compounds seem to be present exclusively in this plant, providing an extra value to these precious red gems.
- Uncommon polyphenols called glucitol-core containing gallotannins (GCGs) in *Acer* L. species, *Acer rubrum* L., and *Acer saccharum* Marsh. that represent the major source of maple syrup, largely used as topping for cakes and sweets. They are formed by galloyl groups attached to a 1,5-anhydro-D-glucitol core (Ma et al. 2016).
- Free gallic acid has also been found in red fruits, *Vitis aestivalis* Michx. and *Vitis vinifera* L., in black tea (about 125 mg/L) and in green tea (about 6 mg/L). It is also present in *Oryza sativa* L. (between 17 and 35 mg/Kg) and in oat flour (*Avena sativa* L.) (Daglia et al. 2014).

25.3 Bioavailability and Metabolism

The development of potential natural therapeutic drugs requires detailed knowledge about pharmacokinetic parameters. The bioavailability of vegetable compound is never free from trouble. It depends by the complexity of phytocomplex that at the same time might be responsible both for an incremented general beneficial effect and for floating absorption of a compound, caused by the concomitant presence of anti-nutrient (i.e., matrix or non-soluble tannins). In fact, many differences could be observed after administration of the pure compound or the whole extract (Jiamboonsri et al. 2015). Given the structure similarity among gallotannins, the most common gallotannins that occur are simplest gallic acid derivatives: tetra- and

penta-*O*-galloyl-glucoses (Kiss and Piwowarski 2016). Data about the bioavailability of some of them are derived from studies on oral administration of mango seed kernel extract in rats (Jiamboonsri et al. 2015). Gallic acid is rapidly absorbed after oral ingestion, raising the maximum concentration in blood after 60 min, with a half-life of 24 min. In humans, the maximum concentration reaches 2 μmol/L, and the half-life is 1 h. No strong differences may be detected between pure compound and extract administration, differently from the two other major metabolites of mango extract: methyl-gallate and PGG. The comparison between their concentrations in rats blood after intraperitoneal injection (35 and 6 μM, respectively) and after oral administration of high dose (under the detectable limit) leads to the conclusion that they might be widely influenced by intestinal permeability and transformed by gut microflora and hepatic metabolism (Jiamboonsri et al. 2015). However, the level of bioavailability in blood of hydrolysable tannins is limited because of the complete hydrolysis in gut (Karas et al. 2017). The bioavailability might also vary between different gallotannins based on the different degrees of solubility. From spectrometric measurements of di-, tri, tetra-, and penta-galloylglucosides, it has been demonstrated that the number of galloyl groups has an inverse relationship with the water solubility, due to the hydrophobic nature of galloyl groups (He et al. 2006).

Although tannins have complex structures and a well-known toxicity against various organisms, they are metabolized and degraded in their derivatives by some microorganisms. As regards gallotannins, they have the simplest chemical structure, and their degradation occurs quite easily by biological enzymatic way through bacteria, yeast, and fungi or by chemical hydrolysis, under acid or base conditions, providing gallic acid and glucose. The main enzyme that play a pivotal role in gallotannins metabolism is tannase that catalyzes, firstly, the cleavage of depside bonds and, secondly, the hydrolysis of ester linkages, thanks to the strong esterase and depsidase activities (Karas et al. 2017). It has been isolated for the first time from strains of *Aspergillus* and *Penicillium*, and nowadays, it is largely used in food industry as stabilizer in various products, as wine and fruit juice (Sarıözlü and Kıvanç 2009). Its action results in the production of glucose and gallic acid that is decarboxylated in pyrogallol by a specific decarboxylase. From pyrogallol it is possible to gain pyruvic acid, which is converted in *cis*-aconitic acid and then in 3-hydroxy-5-oxo-hexanoate before get into Krebs cycle (Karas et al. 2017).

The rate of gut microbial hydrolysis of simple galloyl esters, like methyl-, ethyl-, and propyl-gallate, mono- to hexa-substituted galloyl-β-D-glucoses, and depsides is faster rather than the higher substituted gallotannins, as octa- to dodeca-galloyl-β-D-glucoses. From in vitro studies, miming the low-acid conditions of gastrointestinal tract on mango and strawberry GT-rich extracts, it emerged that higher molecular weight gallotannins are converted into lower weight gallotannins in 4 h, with a concomitant increase of PGG from 200% to 500%, confirming PGG as a transformation product of higher molecular weight gallotannins.

Specifically, Kiss and Piwowarski (2016) revised literature about gallotannins metabolism in humans. They recovered information on urinary gallotannin-deriving metabolites after gallotannin-rich food intake. They found that the most common are pyrogallol-1-*O*-glucuronide, *O*-methylpyrogallol-*O*-sulfate, pyrogallol-*O*-sulfate,

deoxypyrogallol-*O*-sulfate, 4-*O*-methylgallic acid, and 4-*O*-methylgallic acid-3-*O*-sulfate after mango pulp ingestion and catechol sulfate, catechol glucuronide, pyrogallol sufate, methyl pyrogallol sulfate, and pyrogallol glucuronide from strawberries (Kiss and Piwowarski 2016).

25.4 Bioactivities (Animal Aspects)

Gallotannins seem to be involved in a wide variety of mechanisms linked to human health. The explanation of the bioactivity of gallotannins lies in their chemical structure that presents many hydroxyls, with different chemical roles and properties. In fact, phenolic hydroxyls, thanks to their ability in charge stabilization by resonance, are different from sugar core hydroxyls. As belonging to antioxidant class of polyphenols, gallotannins exhibit a strong antioxidant and antiradical activity, deriving by the ability of phenolic OH to donate a hydrogen or transfer an electron to transform into a more stable radical, as shown in Fig. 4. The two mechanisms occur simultaneously, with a prevalence of first way. SAR studies on radical scavenging activity of gallic acid derivatives demonstrated a clear correlation with the number of hydroxyl groups along with steric freedom and the influence of substituents. The presence of a carboxylic group in gallic acid, with respect to pyrogallol, is responsible of the higher antioxidant activity of the first molecule. In fact, it could be explained by the hydrogen donation propensity of this acid group (pKa∼4), together with the presence of an electron withdrawing group at *para* position that makes the corresponding OH more prone to donate hydrogen. Furthermore, the deriving radical O• is stabilize through two intramolecular hydrogen bonds with *ortho*-hydroxyl groups (Badhani et al. 2015). Basing on these criteria and according to Badhani et al. (2015), gallic acid exhibits the highest antioxidant capacity among various polyphenols. Evidences have demonstrated the role of co-planarity of the molecule and hydroxyls, with a better capability in charge delocalization, in the increment of antioxidant potential.

Gallotannins, as previously told, are promptly hydrolyzed in gallic acid and glucose, demonstrating the importance of gallotannins as diet source of precursors of such a powerful antioxidant compound. As regards gallotannins from *Galla*

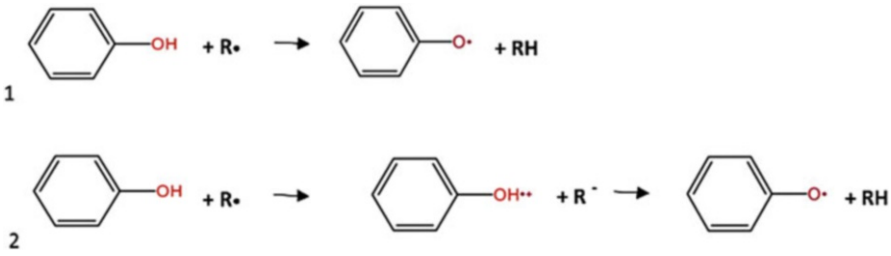

Fig. 4 Antioxidant mechanisms of action of gallotannins. Phenolic OH donates a hydrogen (1) or transfers an electron (2) to transform into a more stable radical

chinensis, studies have demonstrated a clear positive correlation between the degree of polymerization and the antioxidant activity, through different methods (hydroxyl radical scavenging, FRAP, β-carotene-linoleic acid system, and DPPH radical assays), perfectly in line with the evidences about gallic acid (Kiss and Piwowarski 2016). From monogalloylated glucopyranose to deca-galloylated, the antioxidant potential gradually increases, with the exception of di- and tetra-galloylglucopyranose that show an unexpected low activity. It seems that the availability of hydroxyls to hydrogens transfer is reduced by a bond occurring between the galloyl groups. Although some exceptions, the high antioxidant power of gallotannins makes them the perfect substitutes to artificial preservatives in food to prevent oxidation, as well as BHA and BHT, considered as potential carcinogens and severely restricted by Food and Drug Administration.

A comparison between artificial additives and gallotannins has demonstrated the better effectiveness of gallotannins with respect to BHA and BHT (He et al. 2006). The limit of utilization of these compounds as preservatives in food is due to their anti-nutritional effect owing to the interactions with macronutrients that could affect their absorption. The main interactions that occur are both hydrophobic and hydrophilic. The firsts take place between galloyl groups and aliphatic side chains of amino acids, while the seconds between phenolic group of gallotannins and carbohydrates' hydroxyls or polar groups of amino acids, like carboxylic and amino groups. The linkage of gallotannins with polar proteins increases their solubility (He et al. 2006). The capability of gallotannins to interact with biological molecules is responsible of their antimicrobial properties, considered an additional value to their use as food preservatives. The antimicrobial potential is led to the number of pyrogallol rings. Gallic acid, with only one pyrogallol ring, shows a modest antimicrobial activity, but it is able in prevention of biofilm forming by bacteria, in particular by *S. aureus*, *P. aeruginosa*, *E. coli*, and *L. monocytogenes* (Marín et al. 2015).

To translate traditional medicine to modern useful drugs, it is necessary understand the mechanisms that underlie to various bioactivities attributable to gallotannins:

– **Neuroprotective.** Thanks to the hydrophobicity and the antioxidant properties, gallotannins appear as valuable neuroprotective agents (Lu et al. 2006). Oxidative stress and inflammation, with all related pro-inflammatory cytokines release, are the principal events responsible of neurodegenerative diseases occurrence (Daglia et al. 2014). In this context, PGG-rich extracts have an important role, thanks to their lipophilic profile that allows to cross the brain membrane (Lu et al. 2006). Alzheimer disease is characterized by a progressive loss of cognition and memory, and a body of evidences demonstrated the role of accumulation of β-amyloid protein in disease's progression. PGG isolated from *Paeonia suffruticosa* through HPLC analysis showed a great amelioration in the formation of β-amyloid aggregates and also in destabilization of already formed ones. After oral administration of PGG, brain sections of treated and untreated mice have been compared through immunoreactive method, showing a 20% decrease of β-amyloid

aggregates. An improvement in memory deficit was observed in treated mice (Kujawski et al. 2016). This effect might be explained through the antioxidative properties of PGG but also by a new hypothesized method: from in vitro observations on rat neuronal cells, it seems that heme oxygenase-1, a stress response protein, plays a central role in oxidative stress resistance, but further investigations are necessary (Zhang et al. 2009). Although these evidences are derived from PGG of *Paeonia suffruticosa* root extracts (an inedible plant, except for its flowers), Fujiwara et al. (2009) have in vitro demonstrated that this effect is attributed to only PGG molecule that is widely present in other edible plants. Indeed, it is confirmed by Hartman et al. (2006) that observed a great behavior amelioration in mice after pomegranate juice intervention. Treated mice showed an approximately 50% reduction in β-amyloid deposition, compared to untreated mice (Hartman et al. 2006). The neuroprotective activity of gallotannin-rich food might be attributable also to inhibitory activity of tannic acid against β-secretase enzyme, a specific protease that is frequently overactive in Alzheimer disease, with a mitigation of its effects in transgenic mice (Kujawski et al. 2016). The value of gallotannins in the neuroprotection regards also the alleviation of brain damages after ischemia. It is recognized that reperfusion after an ischemic event is associated with severe alterations, as well as inflammation mediator recruitment, radical oxygen species release, and necrosis of brain tissue. Mango leaves-PGG pretreatment to rats subjected to ischemia followed by reperfusion is associated with a mitigation of brain injuries as compared with not pretreated group (Viswanatha et al. 2013).

- **Antidepressant.** The adverse reaction and the tolerance to the modern antidepressant drugs drive to the investigation of antidepressant potential of natural compounds. Among them, gallic acid exhibits a significant activity, proven by forced swim test. The immobility period of mice treated with 60 mg/kg of gallic acid is lower than that of the untreated group, as occurs with target drug, fluoxetine. It acts through a unique mechanism of action, increasing both serotonin and catecholamine levels in the synaptic clefts of the central nervous system. Further interactions with adrenergic, serotonergic, and dopaminergic receptors are also responsible of its antidepressant activity. These results fortify the conclusions of a previous study, conducted on Swiss albino mice, subjected to mild stress. Observing mice behavior under 5, 10, 20 mg/kg doses of gallic acid, Chhillar and Dhingra (2013) concluded that gallic acid reduced immobility time and prevented and restored the stress-induced decrease in sucrose preference. This last evidence might be traced back to the GA-dependent decrement of corticosterone levels in blood. Mild stressed mice present higher plasma nitrite and brain malondialdehyde levels and an increased brain MAO-A activity compared to the control, as well as a decrement of glutathione and catalase activity, as a consequence of the increment of oxidative stress. High doses of gallic acid are associated with the prevention of these alterations (Can et al. 2017).
- **Anti-obesity.** Mango (*Mangifera indica*) seed kernel extract has been identified as a powerful source of anti-obesity compounds. Mice groups following high-fat diet, with and without mango seed kernel extract supplementation, were

monitored. Results showed a reduction in weight gain, fat and visceral fat accumulation, and adipocytes size in treated group, with respect to the control. Hepatic steatosis was observed only in untreated group, while liver sections of treated mice did not reveal histological modifications. These evidences depend on the inhibition power of mango seed kernel extract against intracellular triglycerides accumulation, through the inhibition of the central enzyme of triglycerides biosynthesis. Comparisons with another in vivo study with mango pulp supplementation led to worst results, concluding that mango seed kernel extract contains more anti-obesity bioactive molecules rather than pulp (Kobayashi et al. 2013).

- **Antidiabetic and anti-platelets action.** The increment of obesity is associated with many correlated diseases, first among them, diabetes. In the continuous attempt to find new strategies in diabetes care and maintenance, PGG appears as a valuable lead compound for further therapeutic developments. Gallotannin-rich extracts from *Mangifera indica* L. have been screened in order to identify the antidiabetic compound. Ex vivo studies on the liver and adipose tissues demonstrated the ability of PGG in inhibition of 11β-hydroxysteroid dehydrogenases, a specific oxidoreductase enzyme that plays a central role in the regulation of the levels of glucocorticoids in the body (Mohan et al. 2013). Starting from this result, Mohan et al. (2013) evaluated the activity of PGG on male C57BL/6 mice receiving high-fat diet to induce diabetes. The treatment with different oral doses of PGG (10, 25, and 50 mg/kg) exhibited a clear amelioration of glucose blood levels and fat profile, together with a dose-related decrement of body weight compared to the control (Mohan et al. 2013). In vivo studies on diabetic and obese mice revealed that α-PGG decreases resting blood glucose concentration, with higher glucose tolerance than β-PGG. It could be ascribed to the in vitro demonstrated insulin-mimicking activity of α-PGG that binds to insulin receptors and induces GLUT-4 membrane translocation (Zhang et al. 2009). As insulin receptors are present also on platelets and play an anti-platelets action, Perveen et al. (2011) tested the effects of α-PGG on blood aggregation. Oral treatment with α-PGG (0.02 g/kg) on wild-type mice blocked ex vivo induced platelet aggregation, demonstrating that PGG inhibit platelets activation (Perveen et al. 2011).
- **Hepatoprotective.** As liver damage is correlated with oxidative stress and inflammation, in vivo investigation on the hepatoprotective activity of PGG has been carried on. After treatment with carbon tetrachloride to induce liver injury in rats, a dose of 0.5, 1, and 2 g/kg/day of mango seed kernel extract has been administered. Serum transaminases in treated group showed a decrease stronger than that of rats group treated with the standard drug silymarin, highlighting the hepatoprotective effectiveness of mango seed kernel extract. Furthermore, rats' liver sections presented a remarkable mango seed kernel extract dose-depending improvement in chemical-induced necrosis (Nithitanakool et al. 2009).
- **Nephroprotective.** As hyperoxaluria is associated with an increment of oxidative stress, cell apoptosis, and lipid peroxidation, the antioxidant properties of gallotannins led to investigate their putative effects against kidney stones. In particular, PGG has been tested on hyperoxaluric rats, and it appears to be a

valuable aid in nephrolithiasis prevention, reducing calcium oxalate crystals and oxidative renal cells damage (Lee et al. 2011).

- **Gastrointestinal.** Ulcerative colitis and Crohn's disease are the most common inflammatory diseases that affect the gastrointestinal system. Corilagin, thanks to its immunomodulatory properties, showed therapeutic effect in acute colitis treatment. It has been evaluated by acute colitis model induced by dextran sulfate sodium (DSS) in mice. Intraperitoneal daily doses of 7.5, 15, and 30 mg/kg of corilagin have been associated with an attenuation of colon tissue histological damage and a decrement of pro-inflammatory factors, associated with colitis inflammatory status, by the inhibition of pro-inflammatory action mediated by myeloperoxidase enzyme (Xiao et al. 2013). The limit of this study is represented by the administration route that does not take into account the bioavailability of an oral dose. However, the efficacy of gallotannins in gastrointestinal disease treatment is well recognized as demonstrated by Rajan et al. (2012) who proved the effect of water mango seed kernel extract on castor oil-induced dysentery on Swiss albino mice, confirming its utilization in traditional Chinese medicine. Mango seed kernel extract turned out to be active in altered intestinal motility reduction and in inhibition of prostaglandins, responsible for the unbalanced intestinal permeability in diarrhea (Rajan et al. 2012).
- **Reproductive system.** The treatment of rats with pomegranate juice for 7 weeks has been associated with an improvement of sperm cell density, sperm concentration in epididymis, and sperm quality and motility, thanks to the antioxidant properties of its gallotannins (Türk et al. 2008). Although the responsible molecule of these effects remains uncertain, the same amelioration has been observed also on Leydig cells of male B6 mouse after *Toona sinensis* administration. In this case, the improvement in sperm motility proved not to be induced by an increment of testosterone, confirming the hypothesis of the involvement of antioxidant mechanism tied to gallotannins (Ling Poon et al. 2005).
- **Cholesterol lowering.** Despite its physiological role, high cholesterol level in blood is associated with an augmented cardiovascular risk. The in vivo evaluation of cholesterol lowering potential of gallotannins in pomegranate and mango provides powerful results. In particular, the treatment of albino Wistar rats with an oral dose of mango leaves extract (90 mg/kg for 42 days) showed a reduction in total cholesterol and triglycerides levels in blood (Gururaja et al. 2017). Given the well-known positive role of apolipoprotein E in maintenance of cholesterol and triglycerides levels and in atherosclerosis lesion development, Aviram et al. (2000) tested the pomegranate juice on apolipoprotein E-deficient mice. They observed a 20% reduction in native LDL and oxidized LDL uptake by peritoneal macrophages and by 90% in the oxidation of LDL associated with a decrement of cellular lipid peroxidation and superoxide release by the same cells after pomegranate juice administration. Furthermore, the size of mice atherosclerotic lesions is reduced by 44%, with a concomitant reduction in foam cells number rather than control (Aviram et al. 2000). The anti-atherogenic effects of pomegranate juice, due to its antioxidative properties, have been confirmed by De Nigris et al. (2005). The progression of atherosclerosis is remarkably reduced in

hypercholesterolemic mice, as well as the number of foam cells (De Nigris et al. 2005). In pigs, endothelial damages, induced by dyslipidemia, have been attenuated by commercial skin pomegranate-extract intake (Wang et al. 2018).

- **Antihypertensive.** Evidences of gallotannins-related antihypertensive action are derived from the evaluation of blood pressure in hypertensive mice after intervention with pomegranate juice. Results showed an antagonism toward angiotensin II enzyme, the main regulator in the renin-angiotensin-aldosterone system, involved in blood pressure balance. The mechanism of action is led to the antioxidant properties of gallotannins that avoid the decrement of antioxidant enzymes level, associated with angiotensin II release (Waghulde et al. 2010). Hypertension is correlated with an augmented shear stress, with an underexpression of eNOS and an overexpression of pro-oxidative genes, *p*-JUN and ELK-1, activated by reactive oxygen species. The treatment of mice with doses of pomegranate juice showed an increment of eNOS activity, together with a decrement of oxidative stress-related genes expression (De Nigris et al. 2005).
- **Immunomodulatory.** The term immunomodulatory refers to both immunostimulation and immunosuppression. In autoimmune diseases, an over activity of immune system occurs. PGG appears an effective immunoregulator from in vivo studies. It reduces TNF-α response to LPS stimulus in rats in a dose-dependent manner (Feldman et al. 2001). In contrast, alcoholic mango extract increases humoral antibody and delayed type hypersensitivity in Swiss albino mice, revealing immunostimulant properties, probably attributable to mangiferin (Makare et al. 2001).
- **Antiallergic.** The allergic response is mediated by IgE. PGG exhibits antiallergic properties, due the capability to inhibit B cell signal pathway, with the suppression of IgE production. Ovalbumin-sensitized mice have been utilized in order to evaluate IgE levels after the oral treatment with PGG. It revealed active in the reduction of expression levels of a specific chemokine involved in eosinophil recruitment by IgE receptors. In addition, there is a decrement of the expression of TNF-α and insulin-like growth factor binding protein-3, both involved in IgE production. The antiallergic effect of PGG is enhanced by the immunosuppressive activity of T regulatory cell, which showed an increment, mostly in mice spleen (Kim et al. 2015).
- **Anticancer and anti-inflammatory activity.** The physiopathology of cancer involves several modifications, starting from a primary cancer lesion, with cells over-proliferation and angiogenesis, associated with an inflammatory status. The immunomodulatory, anti-inflammatory, and antioxidative properties of gallotannins put a new perspective on their use as anticancer agents, as demonstrated by many studies. In particular, PGG exhibited in vivo anticancer action against lung cancer, skin cancer, sarcoma, breast cancer, and prostate cancer through different mechanisms that have been in vitro clearly elucidated. However, the way in which PGG exerts the in vivo reduction of tumor remain unclear, because of the lack of in vivo experiments. As regards lung cancer, the intraperitoneal administration of PGG is associated with a dose-dependent decrement of growth, and the maximum dose of 20 mg/kg showed a 91% growth

reduction (Huh et al. 2005). This effect is associated with the suppression of angiogenesis mediated by both the inhibition of pro-angiogenic factors released by COX-2 and the blocking of VEGF binding to VEGF receptor, involved in new blood vessel formation. PGG has been shown a reduction of percentage of skin tumor-presenting mice to about 50% and the average number of tumors per mouse to about 33% (Sieniawska and Baj 2016). A potentiation of immunitary system is at the basis of the effectiveness of PGG or its metabolites against sarcoma. The life expectancy is augmented to 81.9% in sarcoma-bearing mice when they have been treated with intraperitoneal doses of PGG (Zhang et al. 2009). The in vivo efficacy of orally administered PGG on the growth of triple negative breast cancer could be explained through different mechanisms. Zhao et al. (2013) demonstrated the role of both intraperitoneally and orally gallotannins intake in mouse xenograft models in the inhibition of poly (ADP-ribose) glycohydrolase, an enzyme involved in modulation of gene expression (Zhao et al. 2013). Downregulation of estrogen receptors and ErbB family receptors has been proposed as mechanism of action of PGG on this type of cancer by in vivo test on nude mice. The administration of PGG showed a breast cancer regression more pronounced than paclitaxel drug dosing (Zhang et al. 2009). PGG has been revealed a repressor of prostate cancer bone metastasis. It inhibits the expression of metalloproteinase induced by the binding of epidermal growth factor to its receptor in bone, responsible for metastasis. Nude mice treated with 25 mg/kg of PGG three times a week showed lower tumor dimensions and less osteolytic lesions, after prostate cancer cells injection into the bone marrow cavity of tibia, with respect to the control (Kuo et al. 2009). Although PGG showed the highest activity among gallotannins in cancer prevention, also gallic acid and tannin acid demonstrated to be active against cancer. The first has been tested on long-cell lung cancer, after the cell has grafted in mice. The treatment with anticancer drug has been revealed enhanced by the co-administration of gallic acid, with a higher number of apoptotic cells (Kawada et al. 2001). The reduction of tumor growth is also observable in small-cell lung cancer. This efficacy might be explained through the caspase-3-mediated apoptosis induction (Bin-Chuan et al. 2009) and the reduction of inflammatory status, due to the scavenging activity of GA against the reactive oxygen superoxide anion. Its effects on prostate carcinoma xenograft growth have been evaluated on nude mice. They presented a dose-dependent decrement of tumor cell proliferation, associated with a lower angiogenesis rather than untreated mice (Kaur et al. 2009). From in vivo studies, tannic acid appears as a valuable chemopreventive agent. Its activity is explained also when low doses were used: 4.44% of mice receiving tannic acid developed hepatic carcinomas, while 33.3% of cases have been reported in the control group (Nepka et al. 1999). As regards skin tumor progression, tannic acid revealed more potent than GA. It reduces ornithine decarboxylase activity, induced by tumor-promoting agents by 85%. As this enzyme is involved in tumor promotion, it is possible to suppose a putative utilization of tannic acid as anti-promoting agent in many cancer types (Gali et al. 1991).

25.5 Benefits (Human Studies)

In literature, there are several preclinical and clinical studies about the biological effects and pharmacokinetics of simple phenolic compounds; however, human studies are rare, and research findings are controversial on tannin-type compounds (Smeriglio et al. 2017). It has been reported in systematic reviews, and meta-analysis studies that consuming tannin-type compounds found in leaves, fruits, barks, and rhizomes of medicinal plants could avoid chronic diseases (Wang et al. 2014; Turati et al. 2015). Human studies in tannins are on mainly elagitannins and grape seed proanthocyanidins. The outcome of these studies has revealed a reduction in lipid peroxides and enhancement of SOD and CAT activities, GSH and paraoxonase levels, as well as total antioxidant capacity (Horvathova et al. 2014; Deáková et al. 2015). The clinical trials on gallotannin containing medicinal plants were summarized in Table 1.

- **Antimicrobial activity**. The antimicrobial effect of *Hamamelis virginiana* L. distillate and urea (5%) formulation was evaluated in 15 healthy volunteers. The distillate exhibited significant antimicrobial effect on aerobes which are involved in the pathogenesis of atopic dermatitis and intertrigo (Gloor et al. 2002).
- **Anti-inflammatory activity.** In a randomized, placebo-controlled study, vasoconstrictive activity of an aqueous propylene glycol extract of hamamelis was investigated in 30 healthy volunteers. The extract caused a decrease in skin temperature (Diemunsch and Mathis 1987). A mild anti-inflammatory activity of a hamamelis ointment (25 g aqueous distillate/100 g ointment base) was reported in five patients with dermatoses and 22 healthy volunteers. In another study, anti-inflammatory activity of hamamelis distillate was investigated in comparison with chamomile and hydrocortisone (1%) cream and 4 base preparations in two randomized double blind studies in 24 healthy volunteers. The outcome of the study revealed the anti-inflammatory activity of hamamelis PC-cream (Korting et al. 1993). Anti-inflammatory effect of 10% hamamelis distillate lotion was evaluated in 30 healthy volunteers using a modified UVB erythema test model, suggesting that the lotion relieves the symptoms of sunburn inflammation (Korting et al. 1993).
- **Anti-aging activity.** The effect of hamamelis ointment on dry aging skin was investigated in an open-label clinical study in 89 patients at the age of minimum 50 years. Hametum ointment which contains 6.25 mg distillate from fresh leaves and twigs of *H. virginiana* (1:1.6) as the active ingredient was applied to patients were twice daily during 4 weeks. Sebumetric and corneometric measurements and dryness symptoms including tautness, roughness, or itching were recorded. Clinically relevant improvement of skin sebum content and moisture was detected in 4 weeks of application (EMEA 2009).
- **Changes in the gut microbiome.** In a randomized pilot study, potential role of *mango* intake in changes of the gut microbiota, bioavailability of galloyl metabolites, and anti-inflammatory activities in 12 lean and 9 obese (averages

Table 1 Available clinical trials on gallotannin and gallic acid containing natural sources by US National Institutes of Health (www.clinicaltrials.gov)

Number ID	Title	Subjects enrolled
NCT02295293	The effects of *Rhus coriaria* L. on serum lipid levels of patients with hyperlipidemia (SomaghLipid)	74 participants, both sexes, 20–65 years
NCT02891031	The effects of *Rhus coriaria* L. on serum uric acid levels (SomaghUricA)	76 participants, both sexes, 20–65 years
NCT02754089	The effects of *Rhus coriaria* L. on body weight (SomaghWeight)	50 participants, both sexes, 18–60 years
NCT01643096	The effect of thyme and sumac on the thermic effect of food and comparison between warm and cold temperament people	40 participants, both sexes, 18–40 years
NCT02330029	Pinaverium and herbs for irritable bowel syndrome treatment: An onset and offset study (PHIBEST)	800 participants, both sexes, 18–70 years
NCT01641224	A randomized, double-blind, and placebo-controlled study on the treatments of irritable bowel syndrome	800 participants, both sexes, 18–70 years
NCT02511899	Investigation of the influence of careless™, a *Mangifera indica* fruit powder, on microcirculation and endothelial function	10 participants, both sexes, 40–70 years
NCT00935948	Efficacy and safety evaluation of the Imescard compound water smartweed ointment	60 participants, both sexes, 18–70 years
NCT01890070	Study of the nutraceutical properties and health benefits of traditional components of the Mediterranean diet	50 participants, both sexes, 18–75 years
NCT02663492	Transcutaneous electrical Acupoint stimulation for non-small cell lung Cancer patients	300 participants, both sexes, 20–75 years
NCT01501305	Influence of carob and probiotics on acute diarrhea in children	100 participants, both sexes, 3–18 years
NCT02935829	Short-term effects of a carob snack on postprandial glycemic responses and energy intake and satiety	140 participants, both sexes, 18–50 years
NCT02227615	Absorption of mango in healthy individuals	30 participants, both sexes, 20–40 years
NCT02005939	Effect of biophenol-rich pomegranate extract intake on blood pressure, hormones, body composition and quality of life in healthy volunteers. (POM-01Expl)	29 participants, both sexes, 18–65 years
NCT02532101	Modulating effects of oil palm Phenolics in uncontrolled insulin-treated type 2 diabetes mellitus (UNIDOPP)	8 participants, both sexes, 18–70 years
NCT01775384	Polyphenols, exercise, and metabolomics	34 participants, both sexes, 18–55 years
NCT02532088	Modulating effects of oil palm Phenolics in subjects with pre-diabetes (PREDOPP)	268 participants, both sexes, 18–70 years
NCT02328339	Tea and forearm blood flow	20 participants, both sexes, 45–75 years
NCT02273323	Flow mediated dilation in response to black tea (T)	30 participants, both sexes, 18–65 years
NCT02800967	The effects of Aronia juice polyphenols on cardiovascular disease risk (AMARCord)	84 participants, both sexes, 30–50 years

(continued)

Table 1 (continued)

Number ID	Title	Subjects enrolled
NCT03485885	Bioavailability of Maqui Berry extract (MBE) in healthy subjects	12 participants, both sexes, 18–50 years
NCT03214276	Effects of polyphenols supplementation on cycling endurance	48 participants, both sexes, 25–45 years
NCT03805139	Role of Ajwa derived polyphenols in Dyslipidaemias	60 participants, both sexes, 18–70 years

BMI are 22.34 and 33.42, respectively) subjects was evaluated. 400 g of mango was administrated daily for 6 weeks. At the end of 6 weeks, plasma levels of galloyl metabolites enhanced in all subjects, and this was 2.4 times higher in lean subjects. The pyrogallol producing microbiota levels including *Aspergillus oryzae* and *Lactococcus lactis* were significantly lower in obese subjects than in lean subjects. No changes were recorded for *Bifidobacterium* spp. levels. The outcome pointed out that obese individuals do not show the same level of adaptive absorption and metabolism as lean subjects (Kim et al. 2017). *Punica granatum* L. (pomegranate) contains a rich source of biophenols; the most abundant are ellagitannins, anthocyanins, ellagic, and gallic acids. In a study performed on 20 healthy volunteers consuming 1000 mg/day of *P. granatum* extract for 4 weeks, an increase of *Actinobacteria* and *Verrucomicrobia phyla* and a decrease of *Firmicutes* were detected. *Enterobacter*, *Escherichia*, *Lactobacillus*, *Prevotella*, *Serratia*, and *Veillonella* genders also increased, while *Collinsella* decreased significantly. The authors suggested that health benefits may be attributed to the changes in the microbiota (Li et al., 2015).

- **Effect on cholesterol levels:** A two-arm, double-blind placebo-controlled randomized clinical study was carried out on a gallotannin containing medicinal plant *Rhus coriaria* L., also consumed as spice all over the world. Eighty patients with primary hyperlipidemia were randomly assigned and were given 500 mg *R. coriaria* twice daily for 6 weeks. The study indicated significant enhancement in HDL-C and Apo-A1 concentrations with *R. coriaria* supplementation in patients with hyperlipidemia (Hajmohammadi et al. 2018).
- **Antidiarrheal effect.** Antidiarrheal effect of tannic acid-based medical food, Cesinex®, was investigated in six children (18 months to 8 years old) and four adults (55–71 years old) with diarrhea. Two pediatric patients were found to be positive for rotavirus, while in adult patients, no specific cause was found. Diarrheal symptoms were relieved in nine of ten patients receiving Cesinex®. In conclusion, the outcome showed that the tannic acid-based medical food, Cesinex®, was found to be active in managing diarrhea and has a good safety profile (Ren et al. 2011).
- **Effect on irritable bowel syndrome (IBS).** A randomized controlled clinical trial was performed on 1044 adult patients with IBS at 5 hospitals in China, from August 2012 through January 2015. Subjects were randomly assigned (1:1:1) to groups given tongxie (a combination of A macrocephalae, *Paeonia lactiflora*

Pall. along with other herbs), placebo, or pinaverium (50 mg) three times daily for 4 weeks. Significant greater reductions in abdominal pain were detected in patients given tongxie when compared with patients given placebo or pinaverium. It was suggested that tongxie can be considered an alternative therapy for patients with IBS who do not respond well to conventional therapies (Fan et al. 2017).

– **Antiallergic Effect**. A double-blind, placebo-controlled study was carried out to assess antiallergenic activity of Lowal, mixture of 1% benzyl benzoate and 1% tannic acid against dust mites (HDM), Dermatophagoides pteronyssinus and *D. farinae* in carpets. 30 homes (showing more than 400 ng/g of *D. pteronyssinus* and *D. farinae* in carpet dust) of children with HDM sensitization and asthma were enrolled. On day 0 group 1 ($n = 15$) received active treatment, group 2 received placebo treatment ($n = 15$). Two and 8 weeks later, dust samples were collected to determine the amount of mite allergens. After a 2-week washout period, the second treatment was applied as the same manner. A significant decrease was detected for active treatment when compared to placebo treatment after 2 and 8 weeks. It was concluded that slight mite allergen reduction effect was obtained with *Lowal* treatment (Lau et al. 2002)

25.6 Application in Food

Gallotannins are found only in some woody and herbaceous dicotyledons. Cereals are also natural producers of hydrolyzable tannins. *Rhus chinensis* Mill. (Chinese gallnuts) and *Rhus coriaria* L. (sumac) and are rich in gallotannins. β-Glucogallin was reported to be present in *Mangifera indica* L. (mango fruit) pulp (Oliveira et al. 2016). The fruits of sumac are powdered and consumed directly as spice in foods especially in some Mediterranean countries (Lu et al. 2013). Tannic acid is a bitter-tasting substance that binds and precipitates proteins. It is a component found in daily consumed several foods as well as in red wine or unripe fruit and is astringent when taken orally or applied topically. Tannic acid is important in making wine, since it ensures the balance and complexity of wine's palate, color, and aging. Green tea is another source of tannic acid. Tannic acid is also found in the nutgalls which are formed by insects on twigs of *Quercus infectoria* Oliv. Tannic acid is used as a flavoring agent in beverages and foods. Tannic acid was previously used for absorbing poisons in combination with activated charcoal and magnesium oxide. Tannic acid has medicinal applications such as for dysentery, diarrhea, coughs, painful joints, and cancer when taken orally or for bleeding when applied topically. Indeed, it has a protective effect on the skin. It is used for leukorrhea by vaginal application. Tinctures and aqueous extracts are prepared from the fresh leaves of *Hamamelis virginiana* L. by extracting with water and ethanol mixtures in different ratios. Comminuted herbal substances, dry or liquid extracts prepared from the cortex of *Quercus robur* L., and rhizomes of *Potentilla erecta* (L.) Raeusch. are used for medicinal purposes.

25.7 Safety: Toxicity and Side Effects

Polyphenols in food and medicinal plants are presumed to play an essential physiological role in the maintenance of good health. However, due to participating proteins and inhibiting digestive enzymes, tannins are considered as nutritionally undesirable components, resulting in detrimental action on vitamin and mineral absorption. According to published reports, tannins were involved in the incidences of cheek and esophageal cancers, however also recorded as antimutagenic and anticarcinogenic in other studies. Despite their important biological activities, ingestion of large quantities of these compounds may cause some adverse effects.

Tannin toxicity is based on three main mechanisms. The first one is inhibition of microbial enzymes including cellulases, pectinases, xylanases, GPx, laccase, and glycosyltransferase. The second one is their effect on membranes, i.e., inactivation of membrane-bound proteins, inhibition of oxidative phosphorylation, and electron transport system. And the last one is their metal ion chelation effect (Guil-Guerrero et al. 2016). Genotoxicity, short-term, and repeat-dose toxicity studies have been performed on tannic acid. Dietary intake of 2.5% grape seed or grape skin extract caused no-observed-adverse-effect level (NOAEL) in female rats (Serrano et al. 2016). In another study, subchronic oral toxicity of GSE was assessed; the NOAEL of dietary GSE was found to be equal to 1.4 g/kg in male rats and 1.5 g/kg in female rats (Fiume et al., 2014). Moreover, LD_{50} of grape seed and skin extract ingestion was found to be higher than 5 g/kg in rats. Long-term exposure to high concentrations of tannic acid dust may cause lung function changes due to the particles less than 0.5 μm penetrating and remaining in the lung. The symptom is breathlessness and lung shadows. Tannic acid also caused liver tumors in rats following subcutaneous injection (Chemwatch 2008). Despite many studies on the mutagenicity and genotoxicity are available, they are not adequate in evaluating genotoxic risk; therefore, further investigations are highly required (Lluís et al. 2011). Moreover, potential adverse effects cannot be assessed in detail due to lack of information regarding the consumption of these compounds by pregnant and lactating women. In addition, no studies on reproductive toxicology are available (Smeriglio et al. 2014).

25.8 Marketed Products

A Tannic Acid-based Medical Food, **Cesinex**®

Brewtan B, **Brewtan C**, and **Brewtan F** are 100% natural gallotannins specially designed for the brewing industry.

Hametan® cream contains standardized *Hamamelis virginiana* L. extract and is used for wound healing and places in the dermatologic products category.

25.9 Patents

Published patents were searched by using the links (https://worldwide.espacenet.com; https://patents.google.com; https://patentscope.wipo.int/search/en/search.jsf; http://online.turkpatent.gov.tr/EPATENT/servlet/PreSearchRequestManager) with the keywords "gallotannin" and "tannic acid" which have revealed the information presented in Table 2.

25.10 Perspectives

Tannins are natural biologically active components found in edible and medicinal plants. More recently, studies have been conducted on the possible implications of tannin consumption to prevent diseases and provide therapeutic effect by using its derivatives. In in vitro and in vivo studies, tannins were reported to have antimicrobial activity by inhibiting the growth and invasion of fungi, bacteria, and viruses in food plants. Therefore, they can serve as natural regulators of the microbial population in gastrointestinal system. However, biological activity studies as well as activity mechanism studies are still scarce. A preliminary correlation has been reported in clinical studies between potential health effects and dietary intake of tannins, so far. In some epidemiological studies, it was reported that a dietary intake of more than 1 g/day is associated with a reduced many chronic diseases (Sieniawska 2015). Tannins were also reported to display other beneficial activities on immune response, hepatotoxicity, and lipid metabolism. One of the commercially available tannins is tannic acid, which was previously used as antidote against poisons. Nowadays, it is administered topically for the treatment of diaper rash, cold sores, fever blisters, and poison ivy and is also taken orally and applied directly for bleeding, chronic diarrhea, dysentery, bloody urine, painful joints, persistent coughs, and cancer. On the other hand, regarding the safety of gallotannins, potential dangers of diets containing high levels of gallotannins together with food-based polyphenol enrichment and supplements with pure compounds or mixtures have not been studied in detail (Marginǎ et al. 2015). However, the risk related to dietary ingestion of gallotannins is low owing to their poor bioavailability. Nevertheless, the number of clinical studies on gallotannin containing natural sources is not adequate. Further studies on the potential adverse effects that might be associated with high gallotannin consumption are needed. For making certain claims about the health effects in humans, some aspects should be taken into account while conducting clinical studies. Standardized test material should be used in such clinical studies, and the findings should be evaluated by considering individual variations in microbiota. Further clinical studies on human volunteers are warranted to confirm the preliminary findings obtained so far. Absorption and metabolism of tannin-type compounds should be assessed in detail to find out their bioavailability, which is an important

Table 2 Patents on gallotannins

Publication no	Title	Date
WO2015/154074A9	Methods for skin whitening using a gallotannin	October 8, 2015
US4741915A	Protection of foodstuffs from oxidation	May 3, 1988
US4755618A	Recovery of active tannin from *schoene sludge*	July 5, 1988
CA2122746C	High molecular weight gallotannins as a stain-inhibiting agent for food dyes	November 5, 1996
US 6,960.617 B2	Hydrogels having enhanced elasticity and mechanical strength properties	November 1, 2005
US 7,288,273 B1	Gallotannins and ellagitannins as regulators of cytokine release	October 30, 2007
US 8,361,188 B2	Methods for preparing metal and metal oxdenanoparticles	January 29, 2013
US 2003/0078212A1	Pharmaceutical compositions containing poly (ADP-ribose) glycohydrolase inhibitors and methods of using the same	April 24, 2003
US 2006/0058243A1	Methods and compositions for treating diabetes mellitus	March 16, 2006
US 2008/0070850A1	Gallotannins and ellagitannins as regulators of cytokine release	March 20, 2008
US 2010/0129418A1	Method of inducing negative chemotaxis using an ellagitannin or gallotannin	May 27, 2010
US 2015/0307824A1	Clarification method	October 29, 2015
US 2017/0029397A1	Methods for skin whitening using a gallotannin	February 2, 2017
US2018258291 (A1)	Method of forming anti-rust or antibacterial film containing tannic acid derivatives	September 13, 2018
CN108164572A	Method for extracting tannic acid by utilizing herba dendrobii	June 15, 2018
US2018133237A1	Enrichment methods for preparing tannic acid compositions	May 17, 2018
CN107637692A	Application of tannic acid zinc as animal feed additive	January 30, 2018
US6063770A	Tannic acid compositions for treating cancer	May 16, 2000
WO1999004764A1	Tannic acid-polymer compositions for controlled release of pharmaceutical agents, particularly in the oral cavity	February 4, 1999
USOO5994403A	Tannin (tannic acid) treatment of athlete's foot and other fungal infections	November 30, 1999
CN102250159A	Method for extracting and preparing high-purity tannic acid from plant raw material containing tannin	April 28, 2011
CN102127125A	Multielement combined purification preparation process of refined tannic acid series and combined preparation of products	January 5, 2011

(continued)

Table 2 (continued)

Publication no	Title	Date
DE4120296A1	Plant-based combination for use against aids – contains tannic acid, glycosidecpd., bitter substances, ethereal oils, limonene, M-cresol, etc.	June 28, 1991
WO2002069963A2	Compositions comprising ferulic acid, caffeic acid, tannic acid, or ellagic acid and their use for the preparation of a medicament for the treatment of dermatological disorders	March 1, 2001
US4387094A	Contraceptive method and composition containing tannic acid	June 13, 1979
CN1398871A	Tannic acid purifying process	September 10, 2002
CN101838294A	Method for preparing high-purity tara tannic acid	April 7, 2010
US4806526A	Antiallergenic agent	July 11, 1984
CN101260129A	Method of purifying tannic acid	April 12, 2008
CN1450077A	Method for extracting *Polygonum cuspidatum* tannic acid	April 22, 2003
US5198217A	Topical demulcent for viral and inflammatory diseases of the skin	September 24, 1991
US5198217A	Tannic acid preparation for treating burn, bedsore and diaper dermatitis and preparation method thereof	October 12, 2011
CN102140121A	Preparation method of tara industrial tannic acid	January 28, 2010
CN103524571A	Recycling method for preparing tannic acid from walnut residues	October 8, 2013
DE19714567C1	Acaricidal shampoo containing benzyl benzoate and tannic acid	April 9, 1997
WO2000038646A1	Dermatological compositions containing tannic acid and a microbial proliferation inhibitor	December 23, 1998
US6187315B1	Compositions and methods of treating cancer with tannin complexes	March 03, 1995
US6309663B1	Triglyceride-free compositions and methods for enhanced absorption of hydrophilic therapeutic agents	August 17, 1999
US913426A	Process of converting catechin into a catechu-tannic acid	August 9, 1906
CN1398869A	Process of extracting tannic acid from *Geranium wilfordii* root and the use of the extractive	March 9, 2002
CN1589793A	Application of tannic acid berberine in preparation of medicine for treating ulcerative colitis	July 24, 2003
JP2001181869A	Neutralized aqueous solution composition of tannic acid for preventing corrosion of hydrothermal system and producing method therefore	December 7, 1999

issue for their biological effects in clinical studies. In other words, it is essential to conduct bioavailability studies in order to gain a better understanding of the bioactivities of these tannins in human health.

25.11 Cross-References

- ▶ Antioxidants in Diets and Food
- ▶ Dietary Ellagitannins

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Plant Kingdom as Source of Vitamins

26

Céline Rivière and Thierry Hennebelle

Contents

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Abstract

A vitamin is an organic substance necessary, in small quantities, for the correct functioning of the metabolism of a living organism. It is an essential nutrient because this substance cannot be synthesized in sufficient quantity by this organism and thus must be provided through the food intake. Each organism has specific needs: a molecule can be a vitamin for one species and not be it for another. It is the case for example of Vitamin C. Some vitamins are water-soluble compounds, including all vitamin Bs (B1, B2, B3, B5, B6, B7, B9, B12) and vitamin C. Others are fat-soluble compounds and encompass vitamins A, D, E, and K. In this chapter, we have chosen to provide an overview of recent literature data only about vitamins provided exclusively or significantly by plant food, in particular vitamins A (as provitaminic carotenoids), B1, B9, C, E, and K1. Vitamins provided by animal food, such as vitamin D or vitamin B12, will be not discussed in this chapter.

Keywords

Bioactivity · Bioavailability · Diet · Plant kingdom · Food sources · Vitamin A · Vitamin B1 · Vitamin B9 · Vitamin C · Vitamin E · Vitamin K1

26.1 Provitamins A

26.1.1 Bioactive Constituents

The main active form of vitamin A is retinol (Fig. 1) but this compound is rarely found in plant kingdom. Astonishingly, while retinol is produced from plant compounds belonging to the carotenoid group, animal retinoids, and vegetal carotenoids have largely distinct biological roles.

In order to reduce and simplify the field of discussion, this part is strictly restricted to a limited number of carotenoid derivatives that can be attributed provitamin A activity, owing to structural specificities enabling their transformation into it, which requires:

- A mostly linear structure containing numerous double bonds, all encountered as their (*E*) isomer.
- A carbon number that is a multiple of that of retinol, enabling their cleavage into up to 2 retinol moieties: as retinol is a 20C derivative, it would require 40C carotenoids.
- At least one ring system, at an end of the molecule, which is identical to that of retinol. This feature can be referred to as a β-ionone ring system (because it resembles β-ionone, a fragrant degradation product of these compounds) which implies one more conjugated double bond than those present in the linear chain.

While the first two criteria are easily met, being common features in plant carotenoids, the last requirement is much more unusual, making three compounds (Fig. 2) as candidates possibly encountered in the diet at significant levels:

- **β-carotene** (formally β,β-carotene) can yield two retinol molecules.
- **α-carotene**, which is β,ε-carotene – Greek letters here indicate the proper designation for both cycles in the carotenoid nomenclature –yields only one retinol molecule.
- **β-cryptoxanthin**, also called β,β-hydroxy carotene, a xanthophyll (oxygenated carotenoid derivative), also yields one retinol molecule.

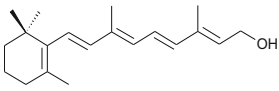


Fig. 1 Structure of retinol

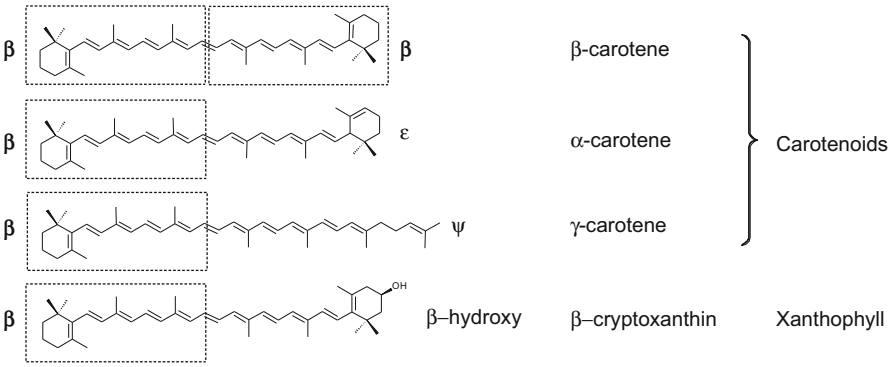


Fig. 2 Carotenoids that are precursors of retinol. β moieties are shown

γ-carotene, the precursor of β-carotene with only one β cyclization, while theoretically a provitamin A, seems to be rare in the diet and has been little studied.

In plants, carotenoids are synthesized in plastids, specially chloroplasts and chromoplasts. Carotenoids are tetraterpenic derivatives produced by the condensation of two geranylgeranyl pyrophosphate molecules (produced from isopentenyl pyrophosphate and dimethylallyl pyrophosphate in the methylerythritol phosphate pathway) by phytoene synthase, considered as the rate-limiting enzyme in the carotenogenic pathway. Phytoene is submitted to desaturation and isomerization reactions to produce the linear compound all-(*E*) lycopene, which can be cyclized and hydroxylated to yield compounds of interest (see Fig. 3). In chloroplasts, most enzymes involved in carotenogenesis have been localized in envelope membranes while, because of their importance in photosynthesis, carotenoids are mostly encountered in thylakoid membranes; in chromoplasts storage forms differ according to the predominant compounds and plant species (Alagoz et al. 2018; Sun et al. 2018). Stabilization of carotenoids involves vitamin E as a radical scavenger protecting them from oxidation and their esterification, for hydroxylated compounds, that is, xanthophylls (Watkins and Pogson 2020).

26.1.2 Bioavailability and Metabolism

26.1.2.1 Bioavailability

Because of their almost strict terpenic origin and hydrocarbon structure, carotenoids are fat-soluble. Their processing in the digestive tract involves their dissolution in the fat phase of the meal. As a xanthophyll, β-cryptoxanthin can be found as esterified derivatives which are hydrolyzed before its absorption, predominantly by cholesterol ester hydrolase (Breithaupt et al. 2002), of pancreatic origin, which differs from retinyl esters (which are hydrolyzed by pancreatic lipase and pancreatic

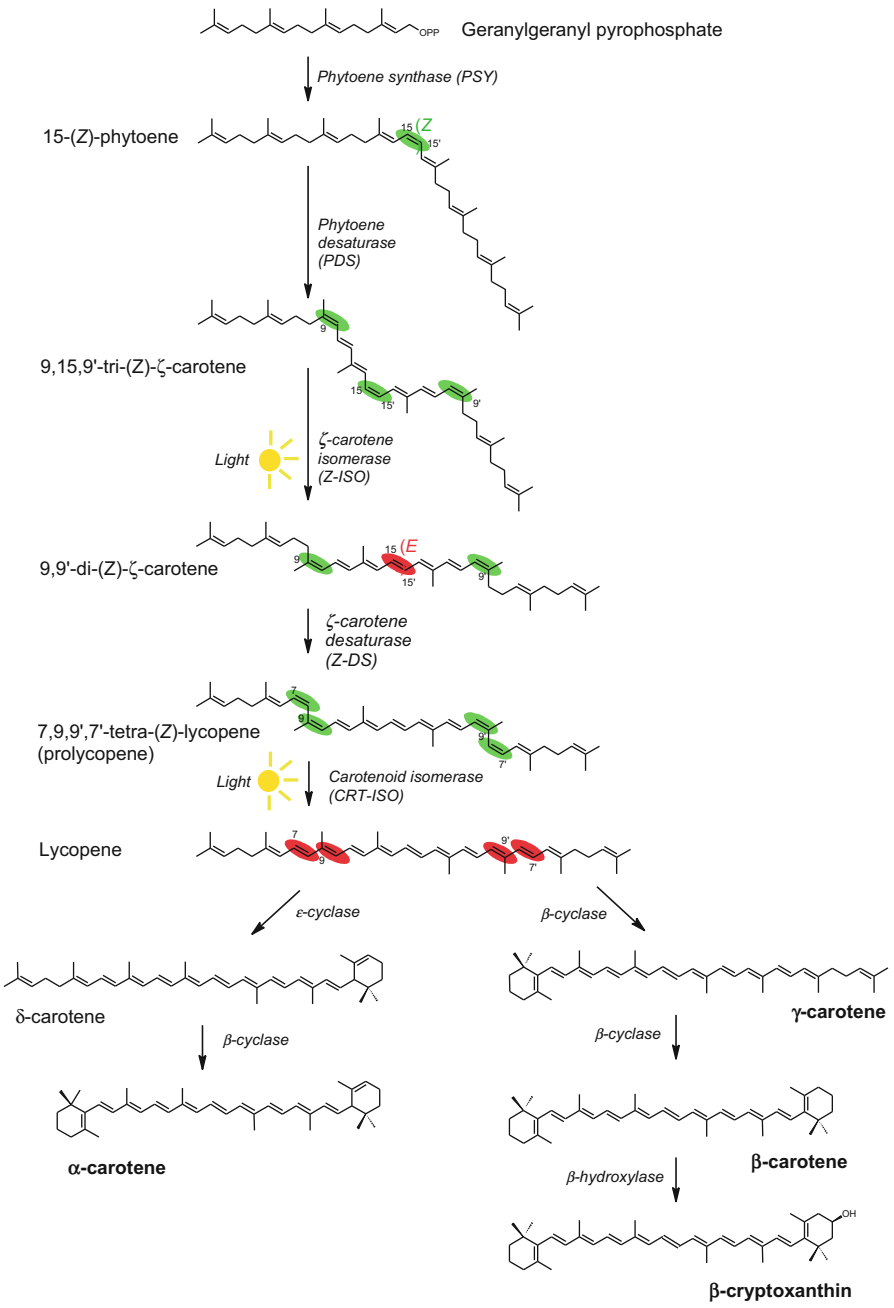


Fig. 3 Biosynthesis of provitamins A in plants

lipase-related protein 2) (Reboul 2013). Free compounds are incorporated to mixed micelles or may be bound to some proteins from the diet (Reboul 2019a). Provitamin A, as other carotenoids, can be absorbed through the apical brush border membrane by a process that has long been attributed to sole passive diffusion, while recent studies have increasingly shown the implication of transporters for lipophilic compounds with low substrate specificity: Scavenger Receptor class B type I (SRBI) (During and Harrison 2007; Borel et al. 2013a), CD36 (van Bennekum et al. 2005; Borel et al. 2013a), and possibly NPC1-like transporter 1 (NPC1L1) (During et al. 2005). The bioavailability of β-carotene was reported with high variability but seems to range between 10% and 30% of the dose typically (Reboul 2019a). β-cryptoxanthin displays better bioavailability, as do xanthophylls in general, which has been attributed to their greater polarity, enhancing incorporation in mixed micelles, enabling location at their outer surface but also to improve solubility in hydrophilic compartments (Burri et al. 2016).

Efflux mechanisms are likely to contribute to the incomplete bioavailability of provitamins A (Reboul 2019a).

26.1.2.2 Metabolism

Even after absorption, only 60–70% of provitamins A are converted into retinoids (Castenmiller and West 1998). This is performed by the cytosolic mucosal enzyme β-carotene 15,15′-dioxygenase BCO1, while minor eccentric cleavage reactions are performed by mitochondrial β-carotene 9′,10′-dioxygenase BCO2 (Amengual et al. 2013). In spite of its name, BCO2 can cleave 9–10 or 9′–10′ double bonds of the carotenoids and xanthophylls (Kelly et al. 2018), yielding apocarotenoids, ionone, and apo-10-carotenal derivatives that depend on the substrate of the enzyme (see Fig. 4). Non-hydroxylated β-10-apocarotenal can be cleaved again by BCO1 and converted to a retinoid. The direct cleavage product is retinal (Leuenberger et al. 2001), which can be further oxidized to retinoic acid (see Sect. 6.3) or reduced to retinol, which can be esterified by lecithin retinol acyl transferase (LRAT) at low doses requiring its binding to intracellular retinol-binding protein, type II (CRBPII); and some acyl-CoA acyl transferases (ARAT), predominantly diacylglycerol acyltransferase 1 (DGAT1) at higher doses (Wongsiriroj et al. 2008).

Retinoic acid exerts negative feedback on its own production in the enterocyte by induction of the transcription factor ISX, repressing both precursor resorption by SRBI and cleavage by BCO1 (Lobo et al. 2010).

Carotenoid derivatives are predominantly secreted through the basolateral membrane of enterocytes, both as free carotenoids and retinyl esters by chylomicrons into the blood via the lymphatic circulation; an ApoB-independent pathway has also been suggested to explain free carotenoid secretion (During and Harrison 2007). It has been attributed to the ABCA1 transporter, which is strongly expressed at the basolateral side of enterocytes (Mulligan et al. 2003) because its expression has been correlated with carotenoid and β-carotene transport (During et al. 2005; Borel et al. 2015) and may enable carotenoid transport by HDL. In both cases carotenoids and retinyl derivatives, once they reach the liver, are stored predominantly in stellate cells (Moriwaki et al. 1988; Blaner et al. 2009), by an LRAT-dependent process for

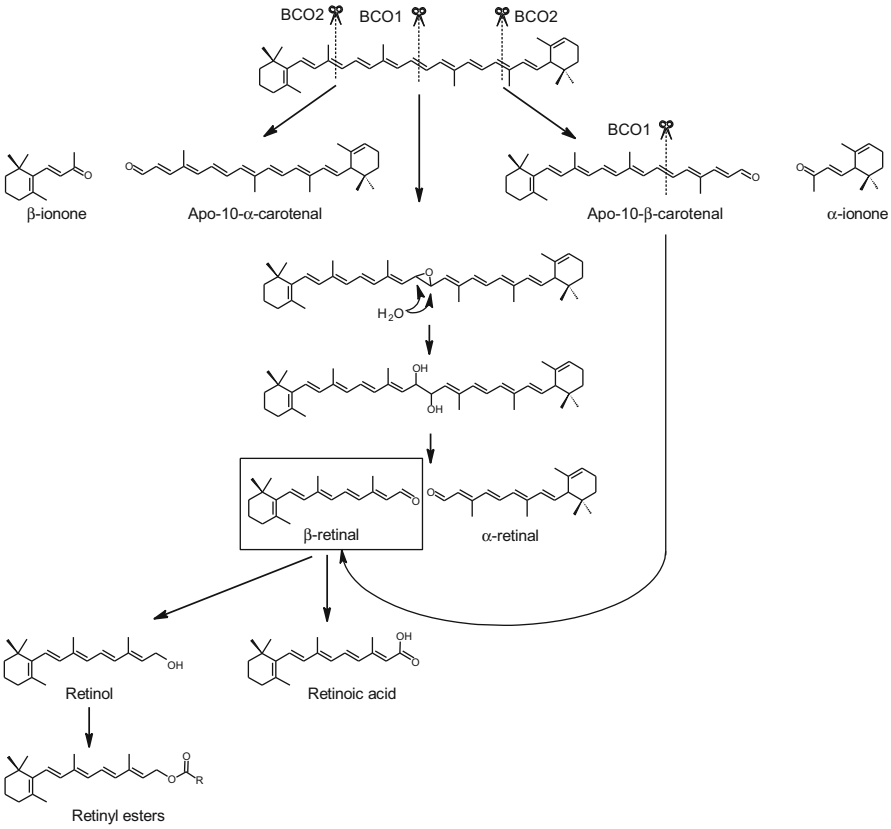


Fig. 4 Conversion of a provitamin A into retinoids in mammals

the latter (Batten et al. 2004; O'byrne et al. 2005). In blood, retinol (obtained by hydrolysis of retinyl esters) is transported by retinol-binding protein (RBP4), which forms a complex with transthyretin, while carotenoids are distributed by LDL. Cellular uptake of retinol relies on STRA6 (Vitamin A receptor, Stimulated by retinoic acid 6 – except in the liver) and RBPR2 (RBP4 receptor-2, primarily expressed in the liver and intestine) (Alapatt et al. 2013), while carotenoids may be transported by LDL receptor and SRBI (Harrison 2019). For a global view of carotenoid transport, see Fig. 5.

26.1.3 Bioactivities

26.1.3.1 General Role

Functions of vitamin A can be attributed to two distinct mechanisms, which explain its general effect and more specifically its importance for vision.

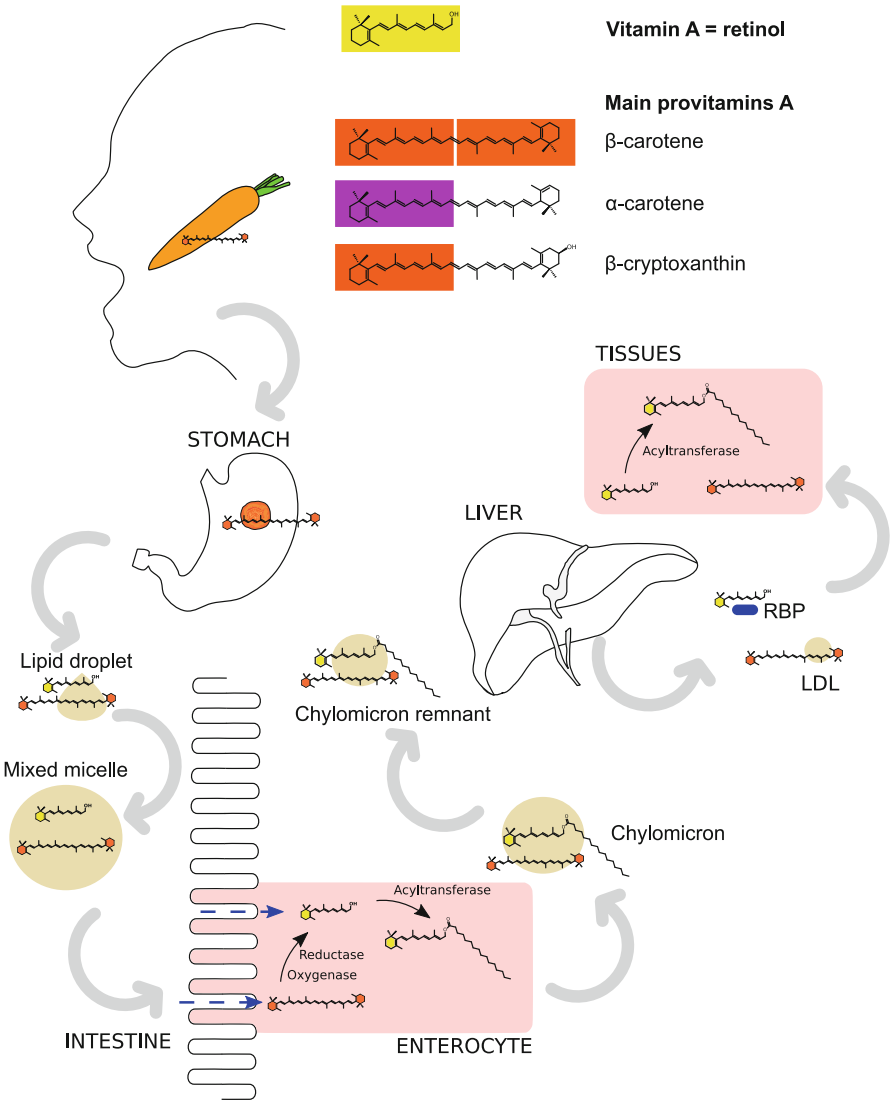


Fig. 5 Global features of vitamin A absorption and distribution in man

Retinoic Acid and Metabolism

Most of the effects of vitamin A are in fact explained by its acidic derivative retinoic acid, acting as a ligand for nuclear receptors that are transcription factors. Various types of such receptors were characterized primarily because of their affinity for retinoids (Fig. 6). Retinoid X receptors (RXR), with three subtypes (α, β, γ), are central because they can act on gene transcription as homodimers or as heterodimers, interacting with other nuclear factors (McGrane 2007), with different potential

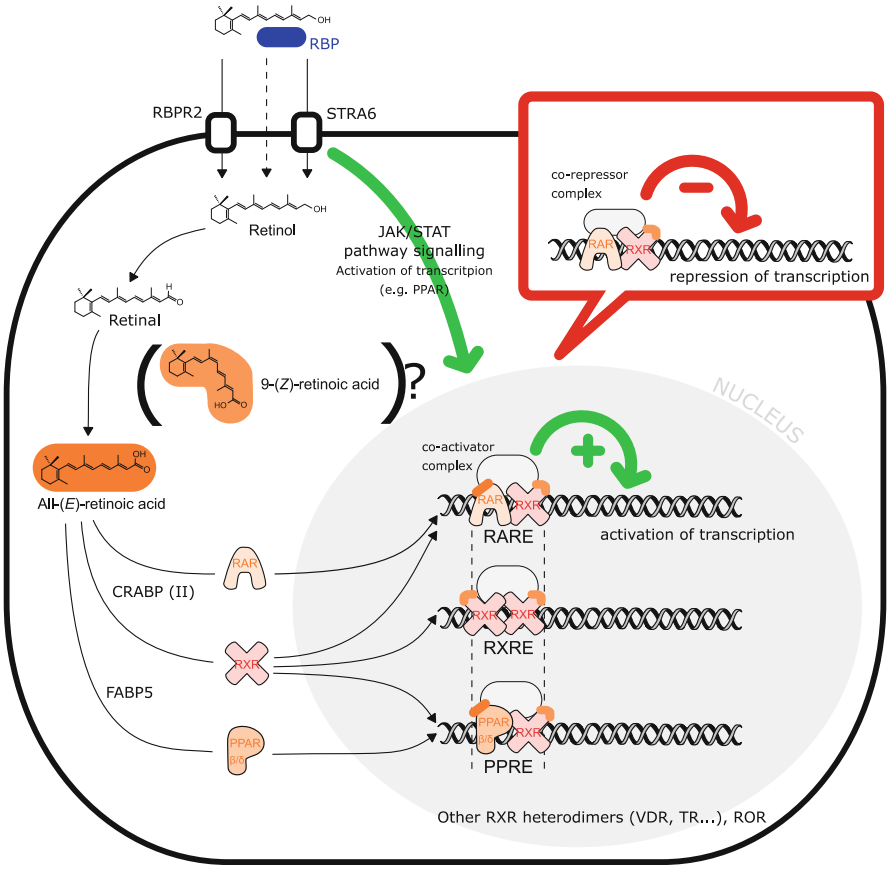


Fig. 6 Effects of retinoic acid on transcription

effects because the activation can be triggered by the RXR ligand, the partner nuclear receptor ligand or both (García et al. 2020).

Two of them have all-(*E*)-retinoic acid as a ligand:

- Retinoic acid receptors (RARα, β, γ) are important in organogenesis and can exert activating or repressing effects (depending on retinoic acid binding or not, respectively) on genes such as *Hox* genes, which are highly conserved for anteroposterior positional patterning information (Marlétaz et al. 2006).
- Peroxisome proliferator-activated receptors (PPAR), particularly of the β/δ type, modulate cell proliferation and differentiation (Michalik et al. 2006).

Another isomer of retinoic acid, 9-(Z)-retinoic acid has been identified as a ligand for RXR and suggested as its natural activator (as shown in Fig. 6) (Allenby et al. 1993). Nevertheless, its biological relevance is unclear (Dawson and Xia 2012). More recently, 9-(*Z*)-13,14-dihydroretinoic acid was detected in several organs and

proposed to be of physiological importance (Rühl et al. 2015), at least in mice, yet its origin remains to be elucidated. RXR ligands are named "rexinoids" and are not necessarily retinoids. Long-chain PolyUnsaturated Fatty Acids (PUFAs), for example, DHA, are RXR ligands (García et al. 2020).

RXR complexes implicate interaction with specific DNA regions called responsive elements, which are named after the distinctive nuclear receptor they interact with: RARE, RXRE, PPRE, or generically RARE. They also require coactivators or corepressors. Retinoic acid transport to nuclear receptors relies on Cellular Retinoic Acid Binding Protein II for RAR and RXR and Fatty Acid Binding protein (FABP5) for PPAR β/δ. The expression of these binding proteins in cell types participates in the pro-apoptotic or proliferative effects of retinoic acid locally (Schug et al. 2007).

Faster, non-canonical (i.e., cytosolic non-RAR-mediated), mostly CRABP(I)-mediated effects on cell cycle have also been reported (Nhieu et al. 2020).

These mechanisms underlie differential effects on cell differentiation, exerting stimulation or inhibition according to specific cell types or conditions (Asson-Batres and Norwood 2020).

In Europe, EFSA Panel on Dietetic Products, Nutrition and Allergies recognized that "Vitamin A has a role in the process of cell specialisation" (EFSA NDA Panel 2009a).

Finally, it is interesting to note that β-cryptoxanthin was identified as a RAR agonist, with distinct effects from all-(*E*)-retinoic acid. This is not directly related to provitamin A activity, as the non-provitamin A xanthophyll lutein displayed similar effect (Matsumoto et al. 2007).

Retinoids in Vision

Vitamin A is involved in the process of vision and light perception (Fig. 7), after its conversion into its aldehyde derivative retinal, which enables light-initiated signal transduction, when coupled to specific transmembrane proteins called retinylidene proteins or opsins s.l.:

- Rhodopsin is the light-sensor of rods, the most numerous photoreceptors in the retina (hence the name "retinal purple"), that permit scotopic (low-light) and peripheral vision.
- Photopsins are located in cones, which are less numerous, but concentrated in the fovea and are dedicated to photopic vision (i.e., vision in well-lit conditions). Three different "cone opsins" with distinct optimal operating wavelength enable color perception.

In both cases, signal transduction involves a (Z) to (E) isomerization and release of retinal, linked to a lysine residue in opsin sequence as a Schiff base, a process called "bleaching." The reversal of this operation requires, after the reduction of retinal by retinol dehydrogenases, the intervention of other retinal cell types in addition to the photoreceptors. The classical visual cycle involves retinal pigment epithelium (RPE) cells in which:

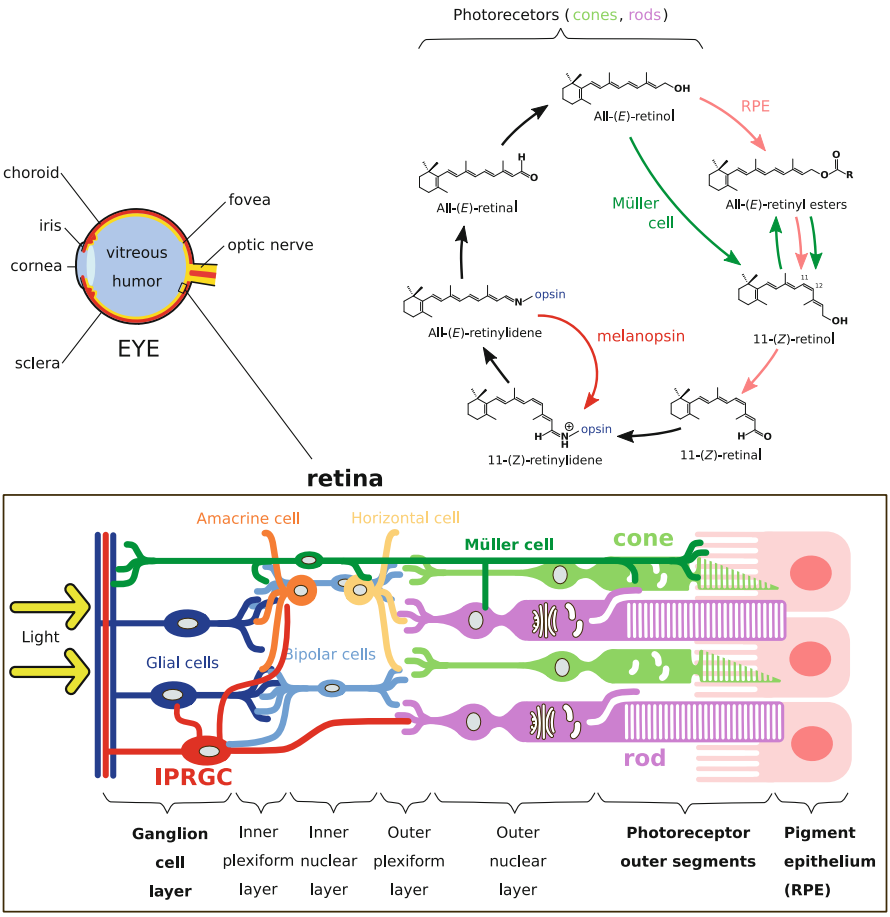


Fig. 7 Vitamin A, eye, and vision

1. All-(*E*)-retinol is converted into retinyl esters by LRAT (Sears and Palczewski 2016).
2. A retinol isomerohydrolase, RPE65 (Retinal Pigment Epithelium-specific 65 kDa protein), both isomerizes the 11,12 double bond and releases free 11-(*Z*)-retinol (Wolf 2005).
3. 11-cis Retinol Dehydrogenase then oxidizes 11-(*Z*)-retinol into 11-(*Z*)-retinal, the active chromophore (Simon et al. 1995).

The linkage between the two parts of this visual cycle (bleaching and all-(*E*)-retinal reduction in photoreceptors; 11-(Z)-retinal regeneration in RPE) appears to rely on Interphotoreceptor Retinoid Binding Protein (IRBP) (Pepperberg et al. 1993).

There is evidence that cones, in addition to this visual cycle, can use another pathway for 11-(*Z*)-retinal regeneration, explaining an increased rate for this process when compared with rods, and maintained signaling even at high light levels (Saari 2012). This cone visual cycle involves Müller cells and differs from the first pathway by the direct conversion of nonesterified all-(*E*)-retinol by dihydroceramide desaturase-1 (DES1) (isomerase-2), presumably (Kaylor et al. 2013) into 11-(*Z*)-retinol, but also, to a large extent, 9-(Z)-retinol. Cellular Retinal-binding Protein (CRALBP or RLBP1) appears to be responsible for 11-(Z) selectivity of the output of Müller cells (Sato and Kefalov 2016). Moreover, cones can uptake 11-(Z) retinol as well as 11-(Z)-retinal, while rods exclusively rely on 11-(Z)-retinal (Wang and Kefalov 2009).

Other retinylidene proteins exist. In the eye, melanopsin is found in Intrinsically Photosensitive Retinal Ganglion Cells (IPRGC) and in low amounts in photoreceptors (Tri Hoang and Yau 2010). It is involved in non-image forming vision, circadian rhythm, and may contribute to mood regulation by light, as mutations have been detected in some cases of Seasonal Affective Disorder (Roecklein et al. 2009; Roecklein et al. 2012; Lazzerini Ospri et al. 2017).

26.1.3.2 Animal Studies

Studies on animals played a prominent role in the study of vitamin A and carotenoids in general, so that a full account might be unreasonable for a book chapter. Some important historical notions can be reminded here:

- While most of the early studies on vitamins were driven by concern over well-identified human diseases (beriberi for thiamin, scurvy for ascorbic acid), it was not the case with vitamin A. The concept of a "fat-soluble A" compound has emerged over the observation that identified macronutrients did not suffice to ensure normal growth of rodents and had to be added some specific fat ingredients. Of course xerophthalmia or night blindness had already been described in Man and treatment by liver had already been suggested several times in history, but the rational route to vitamin A identification hugely relied on animal feeding experiments (Carpenter 2003a, b).
- Retinal pigment and the process of bleaching are very factual observations that could be made only thanks to animal studies, mostly led by the German physiologist Kühne on frog eyes, making it possible to identify the chemical nature of light transduction (Lanska 2010).
- Although the process of retinol identification as "true" vitamin A was quite long, it did not hamper the identification of vitamin A activity of "carotene," neither its conversion to vitamin A in vivo in animals, between 1928 and 1930. Indeed, as the chemical structure was determined more than a decade **before** that of retinol – a surprisingly long delay and obviously a painful experience for researchers of the time) – β-carotene was one of the first vitamin compounds to be characterized (Moore 1930; Pawson 1982).

Noteworthy, not all animal species are able to convert provitaminic carotenoids: in most carnivorous species, this pathway is inefficient or insufficient (Green et al. 2011).

26.1.4 Benefits (Human Studies)

Vitamin A is a recognized immunomodulator. In general, its physiologic affect is to inhibit inflammatory Th1/Th17 and promotes humoral Th2 and Treg response profiles, while this action may be reversed in inflammatory conditions, thus contributing both to tolerance and defense mechanisms, through RXR modulation of RAR effects (Elmadfa and Meyer 2019).

The efficacy of vitamin A supplementation for preventing overall morbidity and mortality in children between 6 months and 5 years of age was confirmed by a meta-analysis. In particular, vitamin A lowers the incidence and risk of mortality from (but not of hospitalization for) diarrhea (Imdad et al. 2017). According to the WHO (2017), diarrhea is the second leading cause of death in children under 5 years old (ca. 525,000 deaths per year) WHO 2017. The efficacy of interventions in babies aged 0–1 or 1–6 months could not be ascertained (Imdad et al. 2016; Haider et al. 2017). A mechanistic explanation for the protective role of vitamin A derivatives in gut infection was recently brought: in a model of salmonella infection in mice, vitamin A stimulates IL-18 production, which acts both on early stages of infection (by promoting cell shedding, i.e., renewal of epithelial barrier and elimination of contaminated cells) and on later stages through Th1 and Th17 responses of immune cells and IFN-γ production, for pathogen clearance. Not surprisingly, this is a RAR-mediated transcriptional effect (Iyen et al. 2020).

No effect was found on mortality from measles, respiratory disease, and meningitis, yet a reduction of incidence was observed for measles. Vitamin A supplementation did not reduce hospitalization for pneumonia, either (Imdad et al. 2017).

Bitot's spots, important early manifestations of vitamin A deficiency in the eye, include infiltration of *Corynebacterium xerosis*, the gas production of which explains their typical foamy appearance (Krishna et al. 2016).

The health claim "*Vitamin A contributes to the normal function of the immune system*" is authorized in Europe (EFSA NDA Panel 2009a, 2011).

Classically, skin and mucosal alterations have been attributed to vitamin A deficiency. Mori (1922) reported xerosis of the trachea and larynx; Wolbach and Howe (1925) described atrophy and keratinization of various glandular organs but found "no striking changes" on skin, with slight atrophy of the hair follicles and sebaceous glands in rats and the sole human case alluded to displayed epithelial modification in various locations, but no specific cutaneous signs.

A frequently mentioned trouble is phrynoderma, which is follicular hyperkeratosis with numerous minute rough-textured papules, yet it is now frequently discussed that this may not be a specific sign of vitamin A deficiency, involving vitamins of the B-complex and/or other nutrients such as essential fatty acids (Heath and Sidbury 2006; Yan and Jen 2012).

On the basis of classical textbooks, the EFSA Panel on Dietetic Products, Nutrition and Allergies stated that

- "*Vitamin A contributes to the maintenance of normal mucous membranes*" (EFSA NDA Panel 2009a, 2010a).
- "*Vitamin A contributes to the maintenance of normal skin"* (EFSA NDA Panel 2009a, 2010a).

Although the description of skin or mucous membranes are not frequently reported in the current medical description of vitamin A deficiency, the interest of retinoids used orally or topically, including natural tretinoin (i.e., all-(E)-retinoic acid) in a variety of dermatological conditions (Chen et al. 2019b) such as acne vulgaris (Kolli et al. 2019), psoriasis (Menter et al. 2020), and eczema (Halioua et al. 2019; Kim et al. 2019), tends to confirm this physiological function.

Resistin-like molecule α (RELMα), a cysteine-rich protein produced by keratinocytes was suggested as the mediator of vitamin A protecting effect against skin infection (Harris et al. 2019).

The possible conversion of topical β-carotene to retinol in human skin was established (Antille et al. 2004).

Vitamin A deficiency is well characterized for causing blindness associated with xerophthalmia and/or keratomalacia (Fig. 8) (Sommer 1998) and the benefit of vitamin A supplementation has been confirmed (Vijayaraghavan et al. 1984). Night blindness is an early manifestation that has intrigued scientists since Antiquity and Galen already recommended liver consumption to treat it (Lanska 2010). Blindness cases are still reported in low-income countries (Daba et al. 2019; Wadhwani and Singh 2020) and xerophthalmia can be observed in Western countries (Lai et al. 2014). Eye defects are both related to retinal-associated vision metabolism and to a loss of transcriptional effects of retinoic acid (Blomhoff and Blomhoff 2006). The possible conversion of circulating β-carotene to vitamin A was demonstrated in human retinal epithelium cells (Chichili et al. 2005).

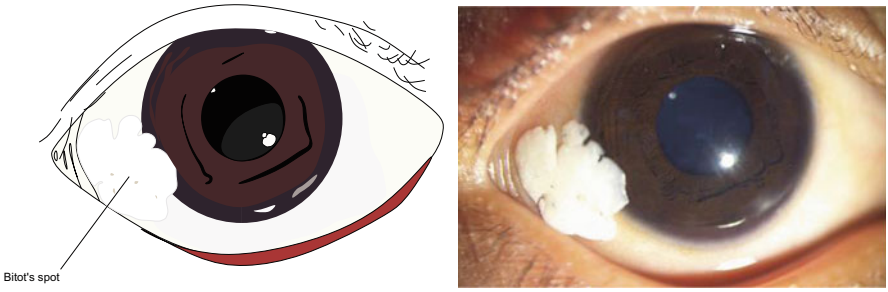


Fig. 8 Bitot's spots in a 4-year-old boy in Indian. (Adapted from Ram and Jinagal 2018)

In Europe, the health claim "Vitamin A contributes to the maintenance of normal vision" is authorized (EFSA NDA Panel 2009a, 2010a).

The positive correlation between vitamin A and iron status has long been evoked and studied. Vitamin A-associated anemia was described as differing from pure iron deficit, with decreased in serum iron, low Total Iron-Binding Capacity, and transferrin saturation, as well as high serum ferritin concentration, because of a lower mobilization of Fe stores, while iron deposits in the liver and spleen. Most clinical trials assessing the benefit of vitamin A supplementation on anemia tend to confirm this observation, yet contradictory data exist. Vitamin A affects erythropoiesis. Its action may also be explained by regulation of ferritin stores and enhanced resistance to infections (Michelazzo et al. 2013).

In Europe the health claim "Vitamin A contributes to normal iron metabolism" is authorized (EFSA NDA Panel 2009a).

As for other vitamins studied here, we focused on European Dietary Reference Values (Table 1) because of their more recent assessment. Vitamin A values are Population Reference Intakes, that is, they could be calculated from an Average Requirement to maintain a concentration of 20 μg retinol/g liver, a threshold suggested by Olson in 1987 (EFSA NDA Panel 2015a). Corresponding provitamin A requirements are discussed in the next part.

Causes for insufficient vitamin A include reduced intake (because of inadequate food supply, alcoholism, mental illness or dysphagia), impaired absorption linked to inflammatory causes (IBD, celiac disease), shortening of the digestive tract (short bowel syndrome, bariatric surgery), and chronic diarrhea. Pancreatic insufficiency is also a possible cause but it does not hinder carotenoid absorption (pancreatic juice is required for retinyl esters hydrolysis). Disordered transport (in abetalipoproteinemia) and reduced storage (in liver disease or cystic fibrosis) can also be observed (Krishna et al. 2016).

Table 1 Dietary reference values of vitamin A (RE) from EFSA, expressed in μg/day

Infants	
0–6 months	–
7–11 months	250
Children	
1–3 years	250
4–6 years	300
7–10 years	400
11–12 years	600
Adolescents	
13–14 years	600
15–17 years	750 (♂) / 650 (♀)
Adults	
18–39 years	750 (♂) / 650 (♀)
Pregnancy	700
Breastfeeding	1300

26.1.5 Applications in Food

The contribution of provitamins A to the presence of retinol in the body depends on the number of provitaminic moieties they contain, but also to their interaction with plant tissue and food matrix (Institute of Medicine 2000, 2001), time spent in the intestine, and the efficiency of cleavage into retinol, submitted to interindividual variations and not restricted to the sole enterocytes (Chichili et al. 2005), which make precise measure tricky. The necessity of a conversion tool for taking provitamins into account was expressed by a Joint Expert Group of FAO and WHO as soon as 1967. Two main methods are used nowadays for the estimation of total retinol available from food, without a clear consensus on the best option:

- RE (retinol-equivalent) = retinol + β-carotene/6 + (α-carotene + β-cryptoxanthin)/12 (FAO/WHO 1967)
- RAE (retinol activity equivalent) = retinol + dietary β-carotene/12 + (α-carotene + β-cryptoxanthin)/24 + β-carotene in oil/2 (US Institute of Medicine 2001)

Table 2 presents plant foods that may be considered rich in vitamin A according to European law (Regulations n° 1924/2006 and 1169/2011) and to the definition of RE used by EFSA. To provide at least 240 μg RE/100 g, they should contain ≥1440 μg/100 g β-carotene.

Amounts in α-carotene and β-cryptoxanthin have been recorded only in the USDA database (https://fdc.nal.usda.gov/index.html), to our knowledge. They were not included in Table 2 because there is scarcely any identified food ingredient that may qualify as rich in vitamin A because of them. The most important remarkable foods providing β-cryptoxanthin are paprika (6186 μg/100 g), butternut squash (3471 μg/100 g), which may be considered as rich in vitamin A for these amounts alone (≥12 × 240 = 2880 μg RE/100 g), and persimmon (1447 μg/100 g), which may qualify as a source of vitamin A on the same basis (≥12 × 120 = 1440 μg RE/100 g). We identified no food ingredient that may be recognized as a source of vitamin A because of its α-carotene content. Butternut squash, again, had the highest amount of this compound with a maximum value of 935 μg/100 g.

Provitamins A like other carotenoids can be quite stable in plants and their availability may be enhanced by cooking (Liu et al. 2004) because heat contributes to their release from their accumulation forms in plastids (Sun et al. 2018). While their measured amounts are little affected by short heating conditions (Khachik et al. 1992), heat as well as light causes isomerization that results in an equilibrium between (E) and (Z) forms (Pesek et al. 1990; O'Neil and Schwartz 1995). This is also observed in fresh foods, to varying extents depending on the species considered (Chandler and Schwartz 1987). The oxidative degradation of carotenoids exists naturally and contributes to food flavor and fragrance but can be enhanced by processing (Vervoort et al. 2013). All these reactions may finally lower vitamin A activity in the product (Zechmeister 1949; Rodriguez-Amaya and Tavares 1992).

Table 2 β-carotene content of plant products which may qualify as rich in vitamin A (ANSES 2017). Only food ingredients with confidence levels A–C were reported. Min–max values, when available, were discarded for clarity purpose

Category	Food	β-carotene (μg/100 g)	RE (μg/100 g) based on β-carotene exclusively
Algae	Bladderwrack (*Fucus vesiculosus*, *F. serratus*), dried	12,400	2067
	Dulse (*Palmaria palmata*), dried	15,700	2617
	Knotted kelp (*Ascophyllum nodosum*), dried	3730	622
	Kombu (*Laminaria japonica*), dried	393,000	65,500
	Sea spaghetti (*Himanthalia elongata*), dried	6620	1103
	Wakame (*Undaria pinnatifida*), dried	104,000	19,333
Fruit	Apricot, dried	2160	360
	Cantaloupe	2020	337
Spices and herbs	Basil, fresh	3140	523
	Chive, fresh	1610	270
	Coriander, fresh	3930	655
	Harissa (chili pepper paste)	4000	667
	Mallow (jute) (*Corchorus olitorius*), dried, powdered	2360	393
	Marjoram (sweet), dried	4810	802
	Parsley, fresh	5050	842
	Sage, dried	3490	582
	Thyme, fresh / dried	2850/2260	475/377
Starchy vegetables	Sweet potato, cooked / raw	12,800/8510	1750/1418
Vegetables	Butternut squash pulp, raw	4230	705
	Cabbage green, raw	5930	988
	Cabbage pak-choi or pe-tsai, raw or cooked	2550–2680	425–447
	Carot dehydrated / raw, frozen / juice / raw / cooked, frozen / canned, water removed / cooked	34,000/12,800/ 9300/8290/ 8200/5330/3340	5667/2133/ 1550/1382/ 1367/888/557
	Chicory "Frisée"	3430	572
	Corn salad, raw	1550	258
	Cress (garden), raw	4150	692
	Dandelion, raw	5850	975
	Lettuce, raw	3640	607
	Lettuce, Roman, raw	5230	872
	Pepper (red), raw / canned, water removed	1620/1530	270/255

(continued)

Table 2 (continued)

Category	Food	β-carotene (μg/100 g)	RE (μg/100 g) based on β-carotene exclusively
	Pumpkin canned, water removed/ cooked/raw pulp	6940/6020/3100	1157/1003/517
	Spinach frozen cooked or raw / canned, water removed / raw / raw sprouts / cooked	7040–7240/5880/ 5630/2710/1610	1173–1207/980/ 938/452/268
	Tomato caviar / pulp	2180/1980	363/330
	Watercress	1910	318

26.1.6 Safety: Toxicity and Side Effects

The toxicity of retinoids is well known, with possible consequences on the liver, hypertriglyceridemia (with possible pancreatitis), headache, skin and mucosal abnormalities, bone resorption, and fractures. Teratogenicity is probably the most feared side effect, with possible craniofacial, cardiac, nervous, and thymic defects (Olson and Goyal 2020). Unfavorable benefit-risk assessment of vitamin A supplementation has been evoked in children under 6 months of age with an increased risk of bulging fontanels (Imdad et al. 2016; Haider et al. 2017).

The toxicity of β-carotene, which has been the most studied provitamin A, is still in inconclusive and the EFSA SCF and NDA Panel could not derive an upper limit for its consumption. Provitamins A have not been found responsible for teratogenicity or fœtal toxicity, to this date (Olson and Goyal 2020). The enhanced risk of lung cancer found in some trials along with high doses of β-carotene was attributed to possible cytochrome or retinoid pathway interactions (EFSA SCF and NDA Panel 2006).

High intake of carotenoids (presumably β-carotene) may lead in some people to yellow-orange coloration of the skin, carotenoderma (or carotenemia) (Yan and Jen 2012).

26.1.7 Marketed Products

β-carotene has been marketed as a drug to prevent vitamin A deficiency, although vitamin A itself is generally preferred. Several studies tend to confirm the possibility of using β-carotene or even foods containing carotenoids instead of retinoids such as retinyl esters to cure vitamin A deficiency, with likely better tolerance (Carlier et al. 1993; Ncube et al. 2001; Vuong et al. 2002). Yet these findings are contested (de Pee and West 1996). The importance of plant source to reach a correct vitamin A intake is admitted (Haskell 2012) but pure β-carotene is not marketed as a curative agent for vitamin A deficiency at present.

Although some drug retinoids have names suggesting parenthood to carotenoids (bexarotene), we identified no carotenoid derivative (bexarotene, tazarotene), their chemical structure do not specifically recall the typical carotenoid structure, and they are not detailed here as they cannot be considered as retinoid derivatives.

Various dietary supplements contain β-carotene as a supply of vitamin A, as it is altogether considered as safer than actual vitamin A. Its origin is supposed to be synthetic in numerous cases but some supplements claim their natural and vegetal origin. β-carotene may also be marketed for preparation of the skin to sun exposure, although it is not clear whether the intended effect relies on the retinoid pathway, sunscreen effect, or only on mild carotenoderma ("suntanning effect"). The traditional prescription of β-carotene in erythropoietic protoporphyria (Urbanski et al. 2016) is in favor of a possible efficacy of such products.

26.1.8 Patents

A variety of patents have been submitted on carotene or cryptoxanthin production. It appears that scarcely any has been dedicated recently to novel uses.

26.1.9 Perspectives

Carotenoids represent an important source of vitamin A, a structurally simple yet fascinating vitamin and their study and uses are intertwined, while the difference between animal and plant origins is more clear-cut than usual in vitamins. The understanding of retinoid pathway and its subtle influence on cell differentiation and protection is obviously still under development even though a lot has been done. Possible beneficial effects of vitamin A and carotenoids on cardiovascular diseases and cancer (Bhatt and Patel 2020) require better knowledge. The feasibility of reaching higher amounts in plants (Watkins and Pogson 2020) with conserved bioavailability is a challenge for the future. A first step would be to define more efficiently its equivalence to retinol, a piece of data that is still elusive.

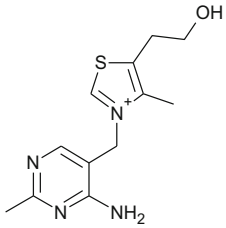
26.2 Vitamin B1: Thiamine

26.2.1 Bioactive Constituents

Thiamine (Fig. 9) is a rather simple vitamin. It contains a pyrimidine and a thiazolium ring.

As usual with vitamin compounds, the biosynthetic origin of this compound is rather mixed and involves a diversity of pathways. In plants, as can be seen in Fig. 10, the pyrimidine derives from 5-aminoimidazole ribotide (Roje 2007), an intermediate compound in purine biosynthesis, through a complex rearrangement that may be similar to that described in some bacterial species such as *Escherichia coli*

Fig. 9 Structure of thiamine



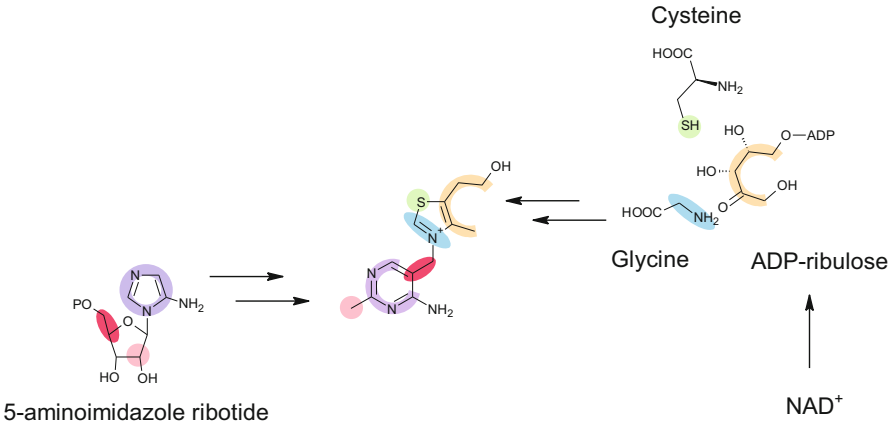


Fig. 10 Likely biosynthetic origin of thiamine in plants

(Begley 1996) and involves S-adenosylmethionine radical-dependent reactions (Coquille et al. 2013).

In contrast, the alkylated thiazole originates mainly in an ADP-ribulose (which is produced from NAD^+), with contributions from two amino acids: glycine, which brings the nitrogen atom as well as its neighboring carbon, and cysteine (a conserved residue in thiamine synthase), which provides sulfur (Goyer 2017). This differs from the bacterial pathway that involves 1-deoxy-D-xylulose-5-phosphate for the backbone, and tyrosine instead of glycine (Begley 1996), but appears to be similar to what was described in yeasts (Chatterjee et al. 2011).

In plants, the pyrimidine and thiazole moieties react as their pyrophosphate and phosphate derivatives, respectively, to afford thiamine phosphate (ThMP). While all the aforementioned steps take place in the chloroplast, ThMP is transported to the cytosol for its conversion into thiamine pyrophosphate (ThDP), the main active form and predominant (up to 90%) vitamer in wild-type plant tissue (Mangel et al. 2017),

through a dephosphorylation-pyrophosphorylation process (Ajjawi et al. 2007; Hasnain et al. 2016).

26.2.2 Bioavailability and Metabolism

26.2.2.1 Bioavailability

Thiamine is predominantly absorbed in its free form, whereas ThMP and ThDP are hydrolyzed by intestinal alkaline phosphatases, according to animal studies (Schaller and Höller 1975; Gastaldi et al. 1988; Rindi et al. 1995). Free thiamine is transported by cation carrier proteins, the specific solute carrier proteins SLC19A2 (ThTr1) and SLC19A3 (ThTr2) (Said et al. 2004) or the more generic organic cation transporter proteins OCT1 and OCT3 (Lemos et al. 2012) in the body, but their respective role in intestinal absorption of thiamine has been diversely reviewed (Brown 2014; Manzetti et al. 2014). ThMP (SCL19A1, RFC1) and ThDP transport exist through anion carriers (Zhao et al. 2002; Nabokina and Said 2012), although their quantitative importance is unclear at this time. Specific transport enables efficient ($\geq$ 95%) absorption of dietary doses of thiamine (Tomasulo et al. 1968; Ariaey-Nejad et al. 1970), but is saturable. Intestinal absorption of thiamine also appears to rely on passive diffusion when high doses are used, with dramatically diminished efficacy (Hoyumpa et al. 1982; Davis et al. 1984).

After oral administration, thiamin reaches its peak concentration in plasma within 1 h and has an elimination half-life of about 150 min (Tallaksen et al. 1993). It is distributed into other compartments and transported into cells, including blood cells : its concentration in plasma (free thiamin + ThMP $\approx$ 16 nM) is 10 times lower than that of whole blood (Thiamine + its mono-, di-, and triphosphate esters $\approx$ 165 nM) (Gangolf et al. 2010), and erythrocytes are usually reported as representing $>$ 80% of blood thiamine (Whitfield et al. 2018). Total thiamine concentration is high in all vital organs. Its liver storage is limited in humans, in comparison with rodents (Gangolf et al. 2010) and liver failure may trigger thiamine deficiency (Butterworth 2009).

26.2.2.2 Metabolism

Thiamine is phosphorylated inside the cells, by thiamine diphosphokinase, into ThDP, which is by far its major form. Pyrophosphokinase can produce ThMP from ThDP, for elimination. To a lesser extent ThDP can also be phosphorylated, becoming ThTP. Some adenylylated derivatives (AThTP and AThDP) can be produced, mainly in fast growing cells (Gangolf et al. 2010). Free thiamine itself and THMP are the main forms encountered in urine, with low amounts of ThDP. Up to 30 degradation products may be found in urine, mostly uncharacterized in humans. Ariaey-Nejad et al. (1970) identified 4-methylthiazole-5-acetic acid and possibly the thiazole moiety of thiamin. A 25-kDa peptide conjugate of thiamine was also reported.

26.2.3 Bioactivities

26.2.3.1 General Role

The most important structural feature in thiamine is the lability of the hydrogen atom between the heteroatoms of the thiazolium. The carbanion thus formed is stabilized by the vicinity of the ammonium, making it an ylid (Fig. 11).

As a coenzyme, ThDP uses this nucleophile to attack carbonyles within various biomolecules, with a constant outcome: the cleavage of one C-C bond next to the carbonyle (see Fig. 12). It is a factor of:

- Dehydrogenase complexes: in these multienzymatic complexes, ThDP is the cofactor of E1 and enables the decarboxylation of ketoacids and the acylation of lipoic acid (the latter, as its amide derivative lipoamide, is a cofactor of the E2 component, which will transfer the acyl residue to coenzyme A). The reactions following this mechanism are summed up in Table 3. All these complexes are located in the inner mitochondrial membrane. The exact mechanism of E1 has been elucidated in pyruvate dehydrogenase by Ciszak et al. (2003). Its general pattern is described in Fig. 12 (green box).
- Transketolase: this enzyme belongs to the pentose phosphate pathway. As described in Fig. 12 (red box), it transfers a C2-unit from a ketose to an aldose in two distinct reactions (Mittschke et al. 2010). Pentose phosphate pathway enables the synthesis of NADPH, a reducing agent in cells as well as that of ribose-5-phosphate, a precursor of nucleotides.
- 2-hydroxyacyl-CoA lyase : this peroxisomal enzyme enables the α-oxidation of 2-hydroxy fatty acids that cannot be catabolized by β-oxidation. It catalyzes their cleavage to produce the corresponding 1C-shortened fatty aldehyde and formate as formylCoA, which involves a nucleophilic attack of the α-keto group by the ylid carbanion of ThDP (blue box in Fig. 12, Casteels et al. 2007).

European Food Safety Authority (EFSA) authorized the health claim "Thiamine contributes to normal energy-yielding metabolism," owing to the dehydrogenases and transketolase roles and the contribution of thiamine in their action. Moreover, this role in energy metabolism (in all cells, including those of the cardiac and nervous systems) has been used to justify two additional claims:

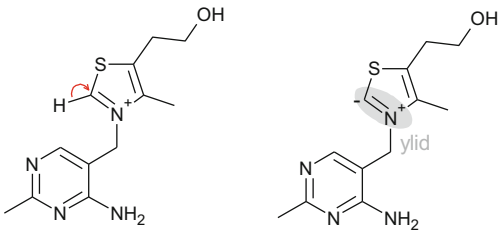


Fig. 11 Formation of the ylid in thiamine

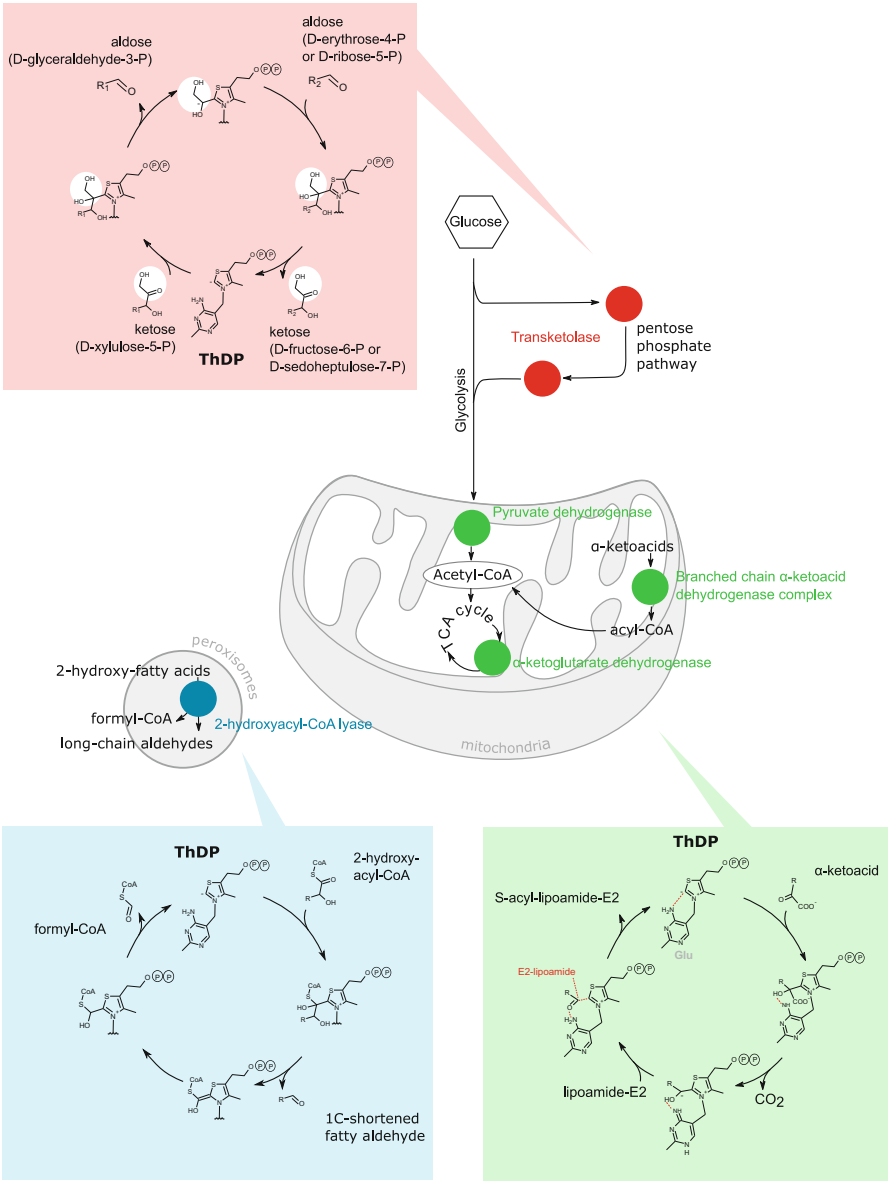
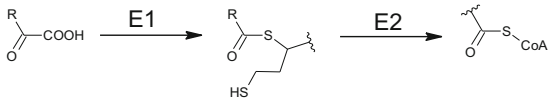


Fig. 12 Enzymatic reactions involving TPP as a cofactor

- "Thiamine contributes to the normal function of the heart" (EFSA NDA Panel 2009b), which may also be linked to the NADPH-mediated antioxidative properties of thiamine.
- "Thiamine contributes to the normal function of the nervous system" (EFSA NDA Panel 2009b), which may involve the importance of thiamine in glucose-

Table 3 Dehydrogenase complexes using ThDp as a cofactor, their substrates and products



Enzymatic complex	Substrate	Structure (R=)	Final product	Role
Pyruvate dehydrogenase	Pyruvate	Me	Acetyl-CoA	Krebs cycle
α-ketoglutarate dehydrogenase	α-ketoglutarate	HOOC	SuccinylCoA	
Branched-chain ketoacid dehydrogenase	α-keto-isovalerate (from deamination of valine)		IsobutyrylCoA	Branched-chain amino acid metabolism
	α-keto-isocaproate (from deamination of leucine)		IsovalerylCoA	
	α-keto-β-methylvalerate (from deamination of isoleucine)		α-methyl-butyrylCoA	

derived neurotransmitters acetylcholine (that requires acetylCoA), glutamate, and GABA (that require α-ketoglutarate) (Butterworth et al. 1986), but also little known ThTP-mediated neuromuscular phosphorylation processes (Bettendorff et al. 2014).

26.2.3.2 Animal Studies

Thiamine is a ubiquitous enzymatic cofactor and is important for numerous animal species. A determinant criterion for the choice of animal assays has been the delay required to achieve observable symptoms with animals on a low-thiamine diet. Historically, birds have been the model of choice that enabled the identification of foods containing thiamine, as well as its purification and structural elucidation. Christian Eijckmann determined the possible location of thiamine in rice bran by its observations of chickens given different meals (Carpenter 2003a). Jansen and Donath (1926) obtained the first pure, crystallized thiamine by means of a bioassay-guided fractionation of rice polishing that relied on the onset time of symptoms in bondol ricebird (cited as "munia maja," presumably *Lonchura maja*, that is, white-headed munia, Fig. 13) fed with polished and washed-off rice supplemented with defined amounts of bran extract fractions (active fractions delayed this onset time which was normally below 15 days). They also used pigeons, which were preferred by other researchers to confirm and continue their work (see for instance Williams et al. 1930).

Fig. 13 Polyneuritis in bondol as depicted by Jansen and Donath

26.2.4 Benefits (Human Studies)

Benefits of thiamine consumption can be easily established by observation of the consequences of a thiamine-depleted diet. This condition has been long known as beriberi without clear insight into its cause until Kanehiro Takaki demonstrated, in the end of the nineteenth century that the use of polished rice alone as food in the Japanese navy was the culprit. More diverse meals resulted in a reduced number of cases, although Takaki's explanation was that beriberi was caused by a protein-deficient disease (Bay 2008). Vorderman, in 1897, observing the diet of prisoners in Indonesian prisons, afforded a hint at the difference between polished and complete rice (Vandenbroucke 2013) (Fig. 14).

Other prominent human studies were those that enabled the definition of what may be accepted as a correct thiamine status. This definition still relies to this day on the measure of erythrocyte transketolase activity, based on papers published in the middle of the twentieth century (Brin 1962; Sauberlich 1967; Wood et al. 1980) because clear cut-off values have not been defined for other markers such as ThDP erythrocyte concentration (EFSA NDA Panel 2016).

Beriberi and more generally thiamine deficiency may cause various symptoms, of which the most prominent are:

- **Neurologic** (thiamine was initially described as antipolyneuritis factor; the name "aneurin" was also used): Mental changes have been admitted as a clear symptom of thiamine deficiency by EFSA, making possible the health claim "Thiamine contributes to normal psychological functions" (EFSA NDA Panel 2010b). Thiamine deficiency or diminished thiamine-dependent enzyme activities have been documented in Wernike-Korsakoff syndrome (Chandrakumar et al. 2018), Alzheimer (Gibson et al. 2016), and Parkinson disease (Lu'o'ng and Nguyễn 2013).
- **Cardiovascular** (cause of death in beriberi; "wet" beriberi was thus named because of dropsy (congestive heart failure) inconsistently occurring in the course of the disease): thiamine deficiency is more prevalent in the heart failure

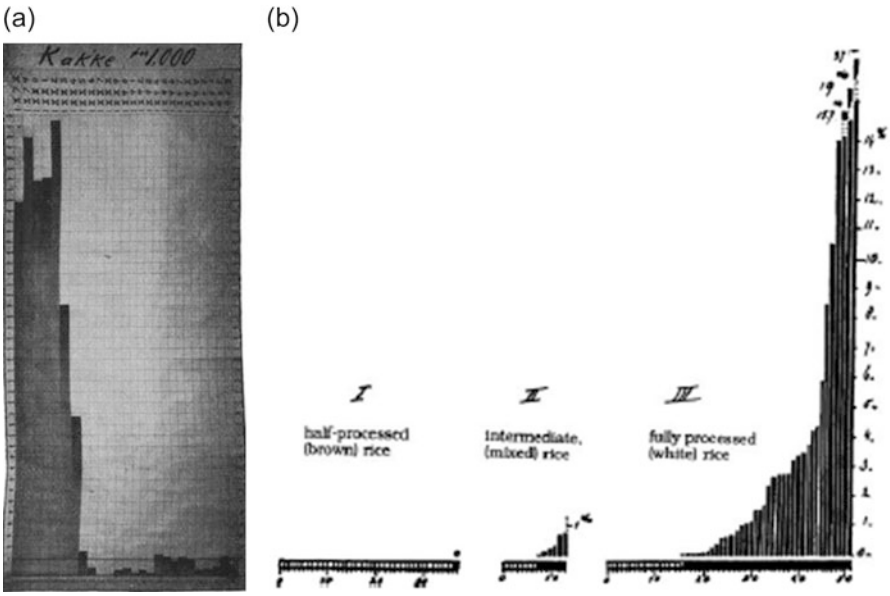


Fig. 14 Historical charts illustrating the effect of dietary intervention on the number of cases of beriberi. (**a**) Takaki's depiction of the dramatic fall in cases thanks to a change in diet (From Funk 1924); (**b**) Vorderman's account of the effect of the processing of rice on the health of prisoners. (From Carpenter 2003a)

population (Jain et al. 2015) and supplementation is beneficial in some cases (DiNicolantonio et al. 2013). Thiamine deficiency may contribute to the pathophysiology of various cardiovascular disorders, especially in the context of diabetes mellitus, because of alternative pathways of glucose metabolism (DiNicolantonio et al. 2018; Eshak and Arafa 2018).

- **Digestive** (anorexia), possibly because of alteration of hypothalamic AMPK activity (Liu et al. 2014). Gastrointestinal symptoms including anorexia may be encounterezd as the only clinical signs of thiamine deficiency (Prakash 2018).

Definition of what may be accepted as correct intakes of thiamine has been a subject of investigation for numerous national and international entities with various outcomes. We focused on EFSA dietary reference values (DRVs), because of their international elaboration, transparency, and recent publication (2016). Data supporting these values and their selection are not discussed here but can be accessed in the corresponding document (EFSA NDA Panel 2016). EFSA set a DRV of 0.1 mg/MJ (0.4 mg/1000 kcal) for all groups of populations. For convenience, estimations of daily requirements in mg/day are presented in Table 4.

Table 4 Dietary reference values of thiamine from EFSA, expressed in mg/day

Population	Male	Female
Infants		
0–6 months	–	–
7–11 months	0.27–0.31	0.24–0.28
Children		
1–3 years	0.33–0.49	0.30–0.46
4–6 years	0.53–0.77	0.49–0.72
7–10 years	0.64–1.02	0.58–0.96
11–12 years	0.86–1.15	0.81–1.06
Adolescents		
13–14 years	0.99–1.32	0.89–1.15
15–17 years	1.14–1.55	0.94–1.20
Adults		
18–39 years	0.96–1.41	0.77–1.13
Pregnancy		
1st trimester[a]	–	0.99–1.05 (1.16)
2nd trimester[a]	–	1.07–1.13 (1.24)
3rd trimester[a]	–	1.17–1.23 (1.34)
Breastfeeding[a]	–	1.17–1.23 (1.34)
≥40 years	0.84–1.35	0.69–1.08

[a]Daily values are provided by EFSA except for pregnancy and lactation values, where recommendations are originally provided as average requirement in addition to values of nonpregnant nonlactating values. Values between brackets correspond to high physical activity level (PAL=2) that was supposed little likely in case of pregnancy or breastfeeding (FAO 2001)

26.2.5 Applications in Food

Thiamine is present in several food categories, including animal-based products. As regards plant sources, it is mainly found in grains (preferably wholemeal grains), legumes, and nuts, as well as spices (which are supposed to be consumed in lesser amounts). Table 5 sums up major plant food sources of thiamine, according to the EFSA Food Composition database (https://www.efsa.europa.eu/en/data/food-composition), selected on the ground of established regulatory thresholds for any food to be considered a source of thiamine (>0.165 mg/100 g) or rich in thiamine (>0.33 mg/100 g).

Thiamine is well-known for its losses in cooking processes. This can be explained by its fragility to various conditions that are summed up in Fig. 15 (Gregory 2017).

It should be kept in mind that, although thiamine disulfide is a possible explanation for reduced thiamine absorption by concomitant polyphenol absorption, its absorption is possible, and it does retain thiamine activity. Naturally occurring reaction products of the sulfhydryl form of thiamine, such as allithiamine, may hinder thiamine detection or quantification, while preserving vitamin status of

Table 5 Thiamine content of plant products which are at least sources of thiamine

Category	Food	Thiamine content (mg/100g)				
Grains		Flour	Groats	Grain or rolled grain	Bran	Germ
	Barley	0.25		0.12 (pearled)-0.15	-	-
	Buckwheat	**0.36**	0.52	-	-	-
	Maize	0.24	-	-	-	-
	Millet	**0.38**	0.26	0.26-0.31	-	-
	Oat	**0.49**	**0.54**	**0.60**	**0.65**	-
	Rice	0.06	-	0.06-0.09 (polished)-0.42 (brown)	-	-
	Rye	0.19-0.35	**0.56**	**0.35**	0.31	-
	Sorghum	0.25	-	-	-	-
	Spelt	0.28-0.51	-	0.30	-	-
	Wheat (wholemeal/brown, Graham flour)	**0.41-0.44**	**0.36**	**0.46**	**0.80**	**1.99**
	Wheat (white/common)	0.12	-	0.31		
	Wheat (durum)	0.18	-	0.18	-	-
Legumes	Beans (Azuki, common, borlotti, broad, mung, kidney ; dry)	**0.40-0.72**				
	Beans (fresh, without pods)	0.17				
	Chickpea (canned or jarred)	**0.36**				
	Lentils (dry)	**0.40**				
	Soya proteins	**0.37 (textured)-0.76**				
	Sprouts (chickpea, common, soyabean)	0.17-**0.37**				
nuts and oilseeds	Almonds (sweet or bitter)	0.20-0.23				
	Cashew nuts, walnuts	**0.38-0.39**				
	Hazelnuts, pumpkin seeds	**0.46-0.48**				
	Linseeds, Macadamia, pine nut kernels and similar	**0.55-0.63**				
	Mustad seeds and similar	**0.54**				
	Peanuts, sesame	**0.97-0.99**				
	Pecans, pistachios, poppy	**0.72-0.78**				
	Sunflower seeds	**1.93**				
Spices and herbs	Caraway fruit	**0.45**				
	Cardamom fruit	0.20				
	Cumin seed	**0.61**				
	Dill seed	**0.42**				
	Fennel seed	**0.41**				
	Fenugreek seed	0.32				
	Garlic and similar, dill leaves	0.16-0.17				
	Juniper berry	**0.38**				
	Thyme	0.22				
	Vietnamese mint	**0.53**				

–: not available; gray box: amount below threshold defining food sources of thiamine in Europe (<0.165 mg/100 g); bold: amount above threshold defining food rich in thiamine in Europe (>0.33 mg/100 g).

consumers (Fujiwara et al. 1954). On the other hand, thiaminase activity, resulting in vitamin losses, may be encountered in foods such as ferns (Fujiwara and Matui 1953), and tannins probably react with thiamine, producing non-vitamin, non-soluble compounds that were not described (Ventura et al. 2013; Yoshida et al. 2012).

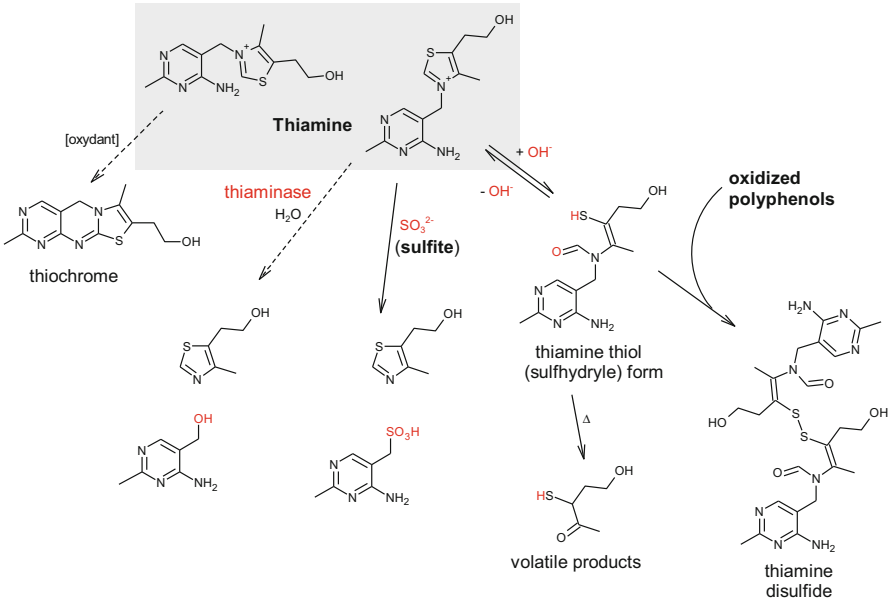


Fig. 15 Degradation reactions described for thiamine

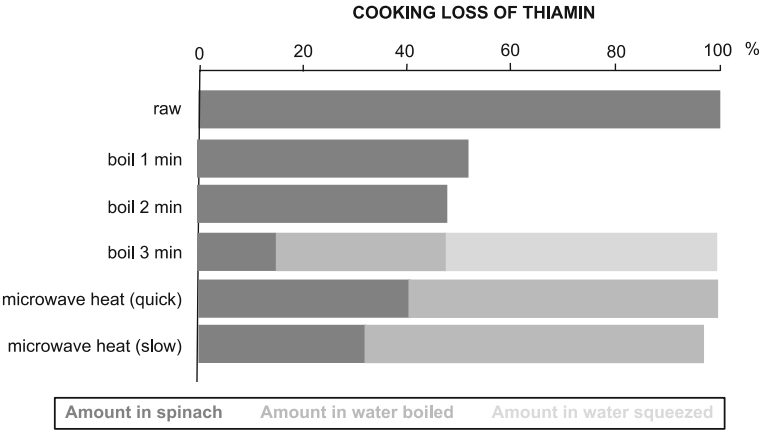


Fig. 16 Changes in thiamine content in spinach cooked by various methods (thiamine content of raw material showed as 100%) according to and adapted from Kimura et al. (1990).

While these degradations may cause considerable thiamine losses in food products or affect significatively its bioavailability, it appears that the most important point is its hydrosolubility, all the more if considering cooking methods at home. This is illustrated by experiments by Kimura et al. (1990), which show that for short cooking times, most of lost spinach thiamine can be found in the water used for boiling it (Fig. 16).

Accordingly, thiamine content tends to diminish dramatically with food processes, in particular cooking. Table 6 shows the impact of cooking in some examples, with calculation of reference dietary intakes (RDI) coverage for suggested portions.

Table 6 Thiamine amount and percentage of RDI covered for defined portions of thiamine-containing plant foods.

		Thiamine amount (mg/100 g)	Portion size[a]	Thiamine amount (mg/portion)	% RDI for one portion
Grain-based products					
Rice grain, parboiled	Raw	0.35	cup 48 g	0.17	15
	Cooked in water	0.09	cup 155 g	0.14	13
	Steamed	0.09	cup 155 g	0.14	13
Pasta wholemeal	Dry	0.54	cup farfalle 81 g	0.4374	40
	Cooked in water	0.10	cup farfalle 107 g	0.107	9.7
Wheat semolina	Raw	0.13	–	–	–
	Cooked in water	0.01	cup 167 g	0.0167	1.5
	Steamed	0.01	cup 167 g	0.0167	1.5
Wheat bread	White (refined flour)	0.13	slice 29 g	0.0377	3.4
	Brown or wholemeal	0.14	slice 29 g	0.0406	3.7
Legume-based products					
Beans	Dry	0.48	–	–	–
	Baked	0.15	253	0.3795	34.5
Chickpeas	Dry	0.45			
	Boiled	0.11	164	0.1804	16.4
Lentils	Dry	0.40			
	Boiled	0.12	198	0.2376	21.6
Nuts and oilseeds					
Cashew nuts	Dry	0.38–0.39	28.35	0.1077	9.8
Sunflower seeds	Dry	1.93	46	0.8878	80.7
Walnuts	Dry	0.39	28	0.1092	9.9
Spices and herbs					
Cumin seed	Dry	0.61	whole tablespoon 6 g	0.0366	3.3
Garlic	Dry	0.16–0.17	3 cloves 9 g	0.0144	1.3
Juniper berry	Dry	0.38	10 berries 1 g	0.0038	0.3

[a]portions are mostly inspired by those proposed by USDA (https://ndb.nal.usda.gov/ndb/search/list)

26.2.6 Safety: Toxicity and Side Effects

There is no data about toxicity from excessive thiamine consumption. As seen above, high doses of thiamine are little absorbed. No toxicological threshold has been established for thiamine (EFSA NDA Panel 2016).

26.2.7 Marketed Products

Thiamine is marketed alone mainly as a curative agent for Wernicke encephalopathy or beriberi, at high doses (e.g., 250 mg). It may have encountered in multivitamin preparation registered as drugs or dietary supplements for specific purposes (asthenia or prevention of B vitamin deficiency in specific conditions such as weight loss surgery). Injectable drugs are available for parenteral nutrition. Marketed thiamine is of synthetic origin.

26.2.8 Patents

A variety of lipid-soluble derivatives have been developed, presumably to ensure bioavailability in cases of high dose administration and enhance restorative action in Wernicke encephalopathy. A part from the naturally occurring allithiamine, some sulfhydryl (benfotiamine, acefurtiamine) or disulfide (sulbutiamine) derivatives have been marketed for ailments in relation with fatigue or neural disorders.

26.2.9 Perspectives

The ongoing interest for thiamine-related disorders in major health concerns such as neurodegenerative or cardiovascular diseases show that there are still good reasons for reflexion about this molecule, its transport in human body, and means to protect it in food. Additionally, plant-sourced (or at least naturally occurring) thiamine may be of interest for dietary supplements as consumers are increasingly fond of natural ingredients.

26.3 Vitamin B9: Folate(s)

26.3.1 Bioactive Constituents

Vitamin B9 is a group of structurally related compounds, which are usually grouped under the term “folate.” Their common structural features consist in a pterin moiety, a *para*-aminobenzoic acid (PABA), which is linked to the pterin by its amino group, through a methylene bridge (forming what is called pteroic acid), as well as a

minimum of one glutamoyl residue, establishing an amide bond with the acyl group of PABA.

While folic acid (i.e., pteroylmonoglutamic acid, Fig. 17) is commonly accepted as the main known form of vitamin B9, it should be kept in mind that it is not a natural compound. Instead, molecules in plants will display two major features of structural variation:

- The pterin moiety is found as reduced forms (dihydrofolate or mainly tetrahydrofolate) which may be substituted by diversely oxidized one carbon units on the N5 and N10 positions (methyl, formyl, formimino) or both (methylene, methenyl); these forms will be discussed in the Sect. 3.3.
- The number of glutamoyl residues varies, affording polyglutamoylated compounds (Fig. 18)

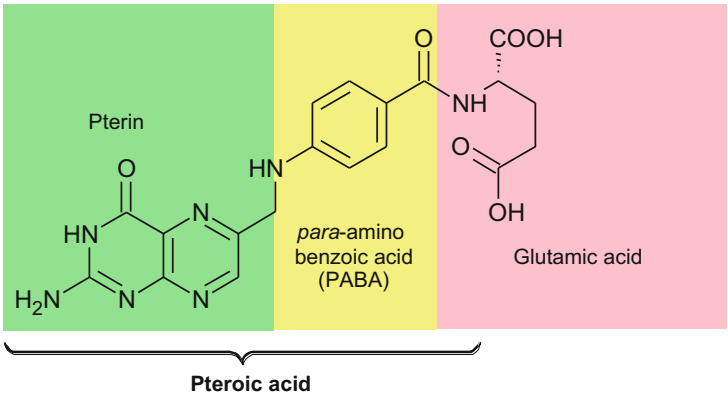


Fig. 17 Structure of pteroylmonoglutamate aka folic acid

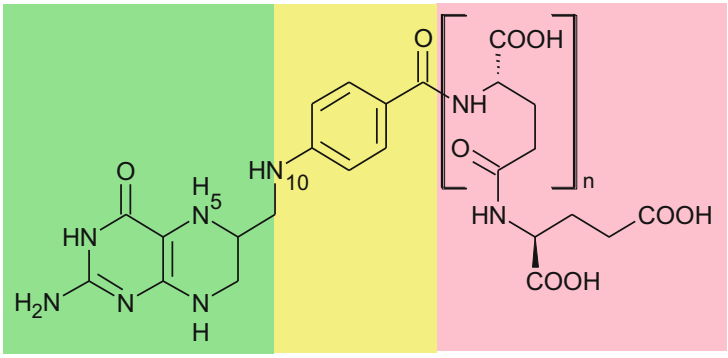


Fig. 18 Structure of naturally occurring folate

Fig. 19 depicts folate biosynthesis in plants (Basset et al. 2002; Ravanel et al. 2001; Sahr et al. 2006). The hydroxymethylpterin moiety is formed as its dihydrogenated state by retroaldolization of dihydroneopterin, the rearrangement product of GTP by GTP cyclohydrolase I (GCHI). The condensation of the pyrophosphorylated form of 6-hydroxymethyl-dihydropterin with PABA, a chorismate derivative, affords dihydropteroate, which may be subsequently glutamoylated and reduced. These biosynthetic steps are common to prokaryotes and eukaryotes, while some specificities exist. In plants they are highly compartmented, with three subcellular contributors; as frequently observed in eukaryotes, steps converting hydroxymethyldihydropterin into dihydropteroate are performed by a same bifunctional enzyme (Mouillon et al. 2002).

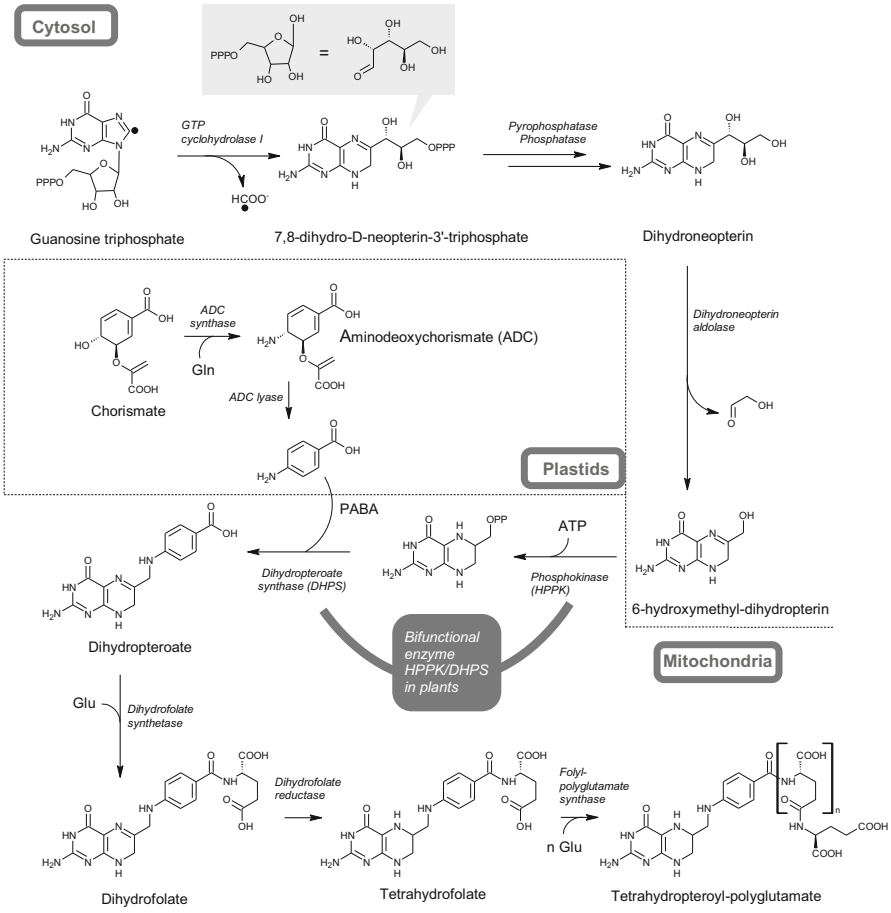


Fig. 19 Folate biosynthesis in plants

26.3.2 Bioavailability and Metabolism

26.3.2.1 Bioavailability

While folates display variations as regards their glutamoylation in food (polyglutamates representing usually more than 50% of total folates), only monoglutamoyl derivatives are absorbed. The hydrolysis of polyglutamates to monoglutamates is predominantly performed by the enzyme folate hydrolase, which is located on the jejunal brush border and encoded by the glutamate carboxypeptidase II (GCPII/FOLH1) gene, in humans and pigs (Bernstein et al. 1970; Chandler et al. 1986). This is not the case in rats where a distinct folate hydrolase (γ-glutamyl hydrolase, γ-GH) is released by pancreatic secretions in the intestinal lumen (Shafizadeh and Halsted 2007). These enzymes operate at different optimal pH values and with different mechanisms, with GCPII sequentially cleaving terminal glutamates and γ-GH being able to catalyze hydrolysis of both internal and terminal glutamates (Chandler et al. 1986; Wang et al. 1986).

Absorption of monoglutamates across the apical brush border is performed by Proton-Coupled Folate Transporter (PCFT) in normal, slightly acidic, conditions, although the Reduced Folate Transporter (RFT), which is present at the same location, may predominate functionally in the case of an increase in pH value (Zhao et al. 2009). RCF operating at suboptimal pH value, colonic transport as well as passive diffusion in the small intestine may contribute to the absorption of folates in some conditions (Zhao et al. 2009; Aufreiter et al. 2009).

26.3.2.2 Metabolism

The involvement of folates in one-carbon metabolism is directly related to its biological activities and interest, so it will be discussed in the following part. Other interesting aspects of folate metabolism are:

- Reconjugation, as polyglutamoylation is used to prevent folate from exiting the cells, making an important difference between a short-lived pool of monoglutamoylated folates and an intracellular polyglutamoylated pool with slow turnover (Stites et al. 1997), located in liver and other tissues (Lin et al. 2004)
- C9-N10 hydrolysis, which is not reversible and accounts for the possible detection of folate breakdown products such as pterin, isoxanthopterin, p-aminobenzoylglutamate, and its acetylated derivative p-acetamidobenzoylglutamate (McPartlin et al. 1993)

26.3.3 Bioactivities

26.3.3.1 General Role

Folate is implicated in a variety of physiological reactions which are strongly intertwined with its biological forms and their interconversion. They are shown in Fig. 20, along with the enzymes involved and spontaneous (nonenzymatic) reactions. The role of tetrahydrofolate (THF) is central:

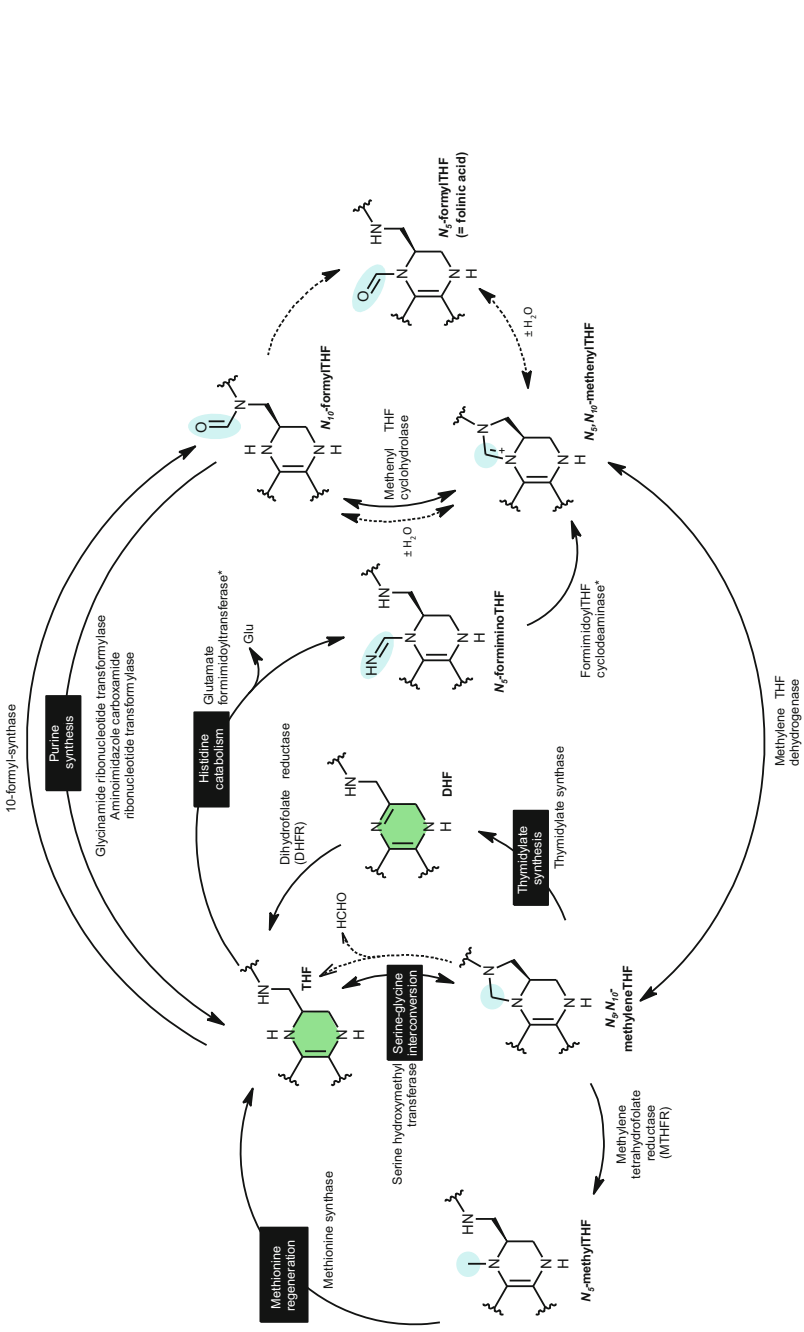


Fig. 20 Interconversion of variants of the pteridine ring of folate. Bold characters: folates; full arrows: enzymatic reactions; dotted arrows: spontaneous reactions; black boxes: biological function

- It can be converted to methylene THF by a serine hydroxymethyl transferase with a one-carbon unit afforded by serine, a dispensable amino acid. Methylene THF is reduced to the more stable N5-methyl THF by methylene tetrahydrofolate reductase (MTHFR). N5-methyl THF is used for transport (its monoglutamate is the major form encountered in plasma (Combs and McCLung 2017); it is also used by methionine synthase for the regeneration of methionine, with the contribution of vitamin B12 (Krebs et al. 1976). Methionine, once adenosylylated, is the universal **methyl donor** in the body. Methylene THF is also the donor of the methyl group in the methylation of uridine monophosphate into thymidine monophosphate by thymidylate synthase (Wahba and Friedkin 1961; Pastore and Friedkin 1962).
- Another contribution of THF is achieved through its receiving a formimino group from formiminoglutamate in the process of histidine degradation into glutamate. Formimino THF is little available as it is immediately transformed by another domain of the same bifunctional enzyme (formiminotransferase-cyclodeaminase) into methenyl THF with an ammonium ion loss (McKenzie et al. 1980; Kohls et al. 2000). THF thus plays some direct part in the **metabolism and interconversion of various amino acids**: serine, glycine, histidine, glutamic acid, and methionine.
- N10-formyl THF can be obtained from THF, directly by 10-formyl synthase or indirectly, after hydration of N5,N10-methenyl THF, a product of methylene THF dehydrogenase. N10-formyl THF is the carbon donor of two transformylases belonging to the purine biosynthesis pathway, implying its own conversion back to THF. Hence, folates are important contributors to the **synthesis of both purines and pyrimidines**, enabling **cell proliferation**. Questions have arisen about the different incorporations of labeled carbon sources (histidine, serine/glycine, and formate) into purines by transformylases leading to the suggestion of possible distinct 10-formyl folate pools by these two enzymes as well as a possibly significant role of N10-formyl DHF as a substrate for aminoimidazole carboxamide ribonucleotide transformylase (Baggott and Tamura 2001, 2015).

All these described enzymatic reactions involve polyglutamoylated forms, which contribute to folate molecular recognition and handling. For instance, substrate channeling (i.e., transfer of the intermediate substrate) in the bifunctional formiminotransferase-cyclodeaminase is optimal with pentaglutamate (Kohls et al. 2000).

In Europe, EFSA authorized four health claims that are related to the roles of folate described above:

- “Folate contributes to a normal function of the immune system” (EFSA NDA Panel 2009c).
- “Folate contributes to normal cell division” (EFSA NDA Panel 2009c).
- “Folate contributes to normal maternal tissue growth during pregnancy” (EFSA NDA Panel 2009c).
- “Folate contributes to normal amino acid synthesis” (EFSA NDA Panel 2010c).

26.3.3.2 Animal Studies

Folate is important for all living organisms, but animals particularly rely on exogenous supply because they lack several enzymes for its biosynthesis. Nevertheless, because the digestive microbiota can synthetize folates, a deficiency is not expected in ruminants, and can be limited or delayed by practices such as coprophagy in rodents.

Historically, the first animal model used for reproduction observed in human folate deficiency was rhesus monkey, leading to the obsolete name "vitamin M" at a time when its chemical identity was not established (Carpenter 2003b).

As microbes provided convenient models for studies on metabolism, animal studies found their major contribution in the comprehension of folate bioavailability mechanisms (Bayes et al. 2019): deconjugation (Bernstein et al. 1970) and transport (Zhao et al. 2009).

26.3.4 Benefits (Human Studies)

As is usual with vitamins, the discovery of vitamin B9 was a direct consequence of the observation of a vitamin B9-deficient diet, making some of its health benefits obvious from the start. Although numerous conditions may be linked to folate deficiency or suboptimal intake, the most important and recognized are:

- **Blood cell defects** – Lucy Wills was the first reporter of "tropical macrocytic anemia" in mothers on what would be later defined as a low folate diet (Carpenter 2003b). Megaloblastic anemias are nowadays known to be related to impaired DNA synthesis, explaining a prominent role of vitamin B9, implicated both in thymidilate and purine de novo synthesis (see Sect. 3.3). Erythroid macrocytosis is an early change in this disease, but neutropenia and thrombocytopenia may develop in severe cases and all fast proliferation cells are affected. Folate-associated megaloblastic anemia is less clearly associated with neurological troubles than those linked to vitamin B12 deficit, nevertheless exhaustion and a depressed mood can be observed (Carmel 2019).
  EFSA authorizes two health claims based on this knowledge:
  "Folate contributes to normal blood formation" (EFSA NDA Panel 2009c).
  "Folate contributes to normal psychological functions" (EFSA NDA Panel 2010c).
  "Folate can contribute to the reduction of tiredness and fatigue" (EFSA NDA Panel 2010c).
- **Impairment of foetal development** – Abnormal folate status has been linked with an increased risk of neural tube defects (NTD). It was at first a suggestion (Smithells 1982), which was subsequently confirmed at high doses (4 mg) and in multivitamin preparation (MRC Vitamin Study Research Group 1991), then at lower doses (Czeizel and Dudás 1992) and as a single ingredient. Other birth defects were supposed to be related to low folate status, with no clear evidence to this day (De-Regil et al. 2015). EFSA authorized the following health claim:

"Supplemental folate intake increases maternal folate status. Increasing maternal folate status contributes to the reduction of the risk of NTD"
if 400 µg supplemental folate is consumed daily for at least one month before and up to three months after conception. (EFSA NDA Panel 2013a)

Folate status in Man can be assessed by folate concentration measurement in serum, where it depends on short-term changes, or in erythrocytes, where it is considered to reflect tissue stores. In both cases analytical methods include microbiological methods relying on the stimulation of the growth of bacteria (generally *Lactobacillus rhamnosus*) by folates, competitive binding to proteins (immunological methods, most of which rely on ligand-labeled folates and chemoluminescence) or liquid chromatography, noteworthily isotope dilution LC/MS/MS (Pfeiffer et al. 2009).

The role of folate in methionine synthesis has directed attention toward indirect consequences of folate deficiency as a biomarker: a defect in methionine regeneration by methylation of homocysteine leads to **hyperhomocysteinemia** (Fig. 21), which has been linked to various conditions including cardiovascular diseases, cancer, and Alzheimer's disease (Kim et al. 2018). The admitted beneficial effect of correct homocysteine remethylation led EFSA to authorize the health claim "Folate contributes to normal homocysteine metabolism" (EFSA NDA Panel 2009c). Nevertheless, the correction of hyperhomocysteinemia by B-group vitamins has not been shown to reduce cardiovascular events, cancer risk, or global mortality (Martí-Carvajal et al. 2017).

More recently, attention was drawn to cystathionine, an intermediate product of cysteine synthesis from homocysteine by vitamin B6-dependent transsulfuration. Elevated plasma cystathionine was found to be correlated with stroke and global mortality in the context of coronary heart disease (Dhar et al. 2018, 2019).

Dietary reference values provided by EFSA (2014) are reported in Table 7. Population Reference Intakes (PRI) calculated from Average Requirements based on biomarkers of folate status could be proposed except in the cases of neonates <6 months and of pregnancy. In the latter, an Adequate Intake based on a study where the intake was sufficient to maintain folate status was observed (Caudill et al. 1997) while the possibility of reaching the same result with lower doses cannot be ruled out. In comparison with other vitamins, the calculation of PRI involved higher coefficient of variation, taking heed of numerous genetic polymorphisms (e.g., MTHFR) possibly increasing requirement for folates in some people.

Other causes for increased requirement in folates may include drug therapy with products described in part 7, but also metformin or phenytoin, alcohol consumption, and various causes of decreased intestinal area or efficiency such as bariatric surgery (McNulty and Pentieva 2009).

26.3.5 Applications in Food

The first successful treatment of folate deficiency was quite immediately proposed by Lucy Wills: it was the famous yeast extract Marmite®. At the time the

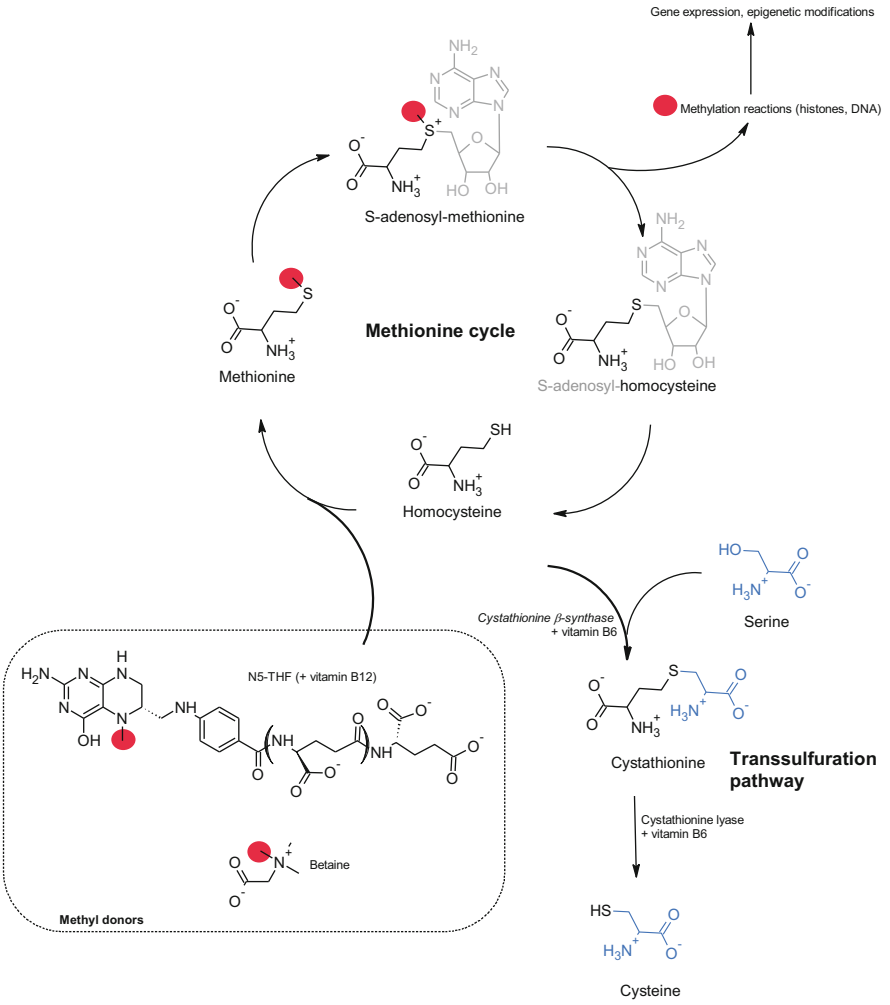


Fig. 21 Methionine cycle and transsulfuration pathway

molecular basis of the disease was not known and the proof of concept was challenged but Wills confirmed the successful treatment of correctly diagnosed pernicious anemia of pregnancy or tropical macrocytic anemia (Fig. 22, Wills 1933).

Folates are present in various foods, noticeably in liver and yeast products. The presence of folates in various plant products is reported in Table 8. We focused on foods that could be defined as "rich in folates" according to European law (Regulations n° 1924/2006 and 1169/2011), that is, ≥60 μg/100 g. Mushrooms and spirulina are also non-animal foods rich in folates.

In spite of their original success as food ingredients, dietary folates were found to have flaws for their intended reproducible use as curative agents in medicine:

Table 7 Dietary reference values of folate from EFSA, expressed in mg/day.

Infants	
0–6 months	-
7–11 months	80
Children	
1–3 years	120
4–6 years	140
7–10 years	200
11–12 years	270
Adolescents	
13–14 years	270
15–17 years	330
Adults	
18–39 years	330
Pregnancy	600
Breastfeeding	-

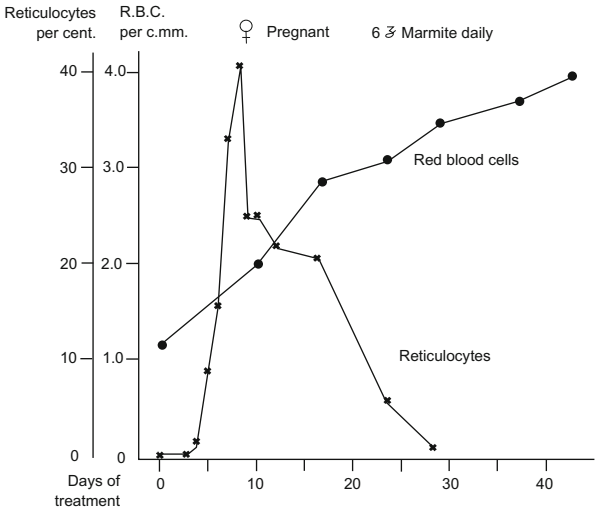


Fig. 22 Marmite yeast extract curative effect on macrocytic anemias as reported by Lucy Wills (right)

- Their diversity, making it difficult to quantify them; Table 9 shows a transcription of several recent attempts at folates in different plant foods: N5-methyl THF is generally the main compound but formyl vitamers can be present in significant amounts or even predominate, for example, in pak-choi.

- Their lack of stability. Fig. 23 sums up reactions described for the degradation of major vitamin B9 vitamers in food, N5-methyl THF, N10-formyl THF, and THF. The oxidation of the tetrahydropyrazine ring is reversible but other oxidative reactions are possible (Blair et al. 1975). Breakdown products are also created by

Table 8 Folate content of plant products which are rich in folates (ANSES 2017). Only foods with confidence levels A–C were reported

Category	Food	Total folate (μg/100 g) (min–max values when available)
Algae	Carragaheen (*Chondrus crispus*), dried	866
	Dulse (*Palmaria palmata*), dried	92
	Kombu (*Laminaria japonica*), dried	127
	Wakame (*Undaria pinnatifida*), dried	237
Fruit	Strawberry, raw	72
	Raisins (dried)	60
Grains and grain-based products	Amaranth, seed, raw	82
	Biscuit, vitamin-guaranteed or vitamin-enriched	140–212
	Breakfast cereals, enriched (mean food)	177
	Breakfast cereals (various), non-enriched	200
	Buckwheat flour	67
	Corn (sweet), preserve, juice removed	75
	Malted powder for drink, enriched	219
	Quinoa, raw	184
	Wheat bran	110
	Wheat durum, pre-cooked, whole, raw / cooked (microwave meal)	331 / 118
	Wheat flour	60
	Wheat germ	143
Nuts and oilseeds	Almonds with skin	94
	Flaxseed	72–94
	Hazelnuts, dried / grilled	121 / 88
	Peanuts / grilled / peanut butter	90 / 67–97 / 70
	Pine nuts, raw	80
	Pistachios, grilled, salted	94
	Poppy seeds	82
	Sunflower seeds, dried	237
	Walnut kernels, dried / fresh	120 / 66
Legumes	Broad beans, dry / fresh / cooked	423 / 122 / 81
	Butter beans, raw / preserve (water removed)	68 / 66
	Chickpeas, dried / cooked	369 / 84
	Chickpea flour	437
	Coral lentils, dried	124
	Falafel (chickpea and/or broad bean), fried	105
	Kidney beans, dried	121
	Lentil, dried / cooked / germed	117–257 (35–596) / 181 / 100

(continued)

Table 8 (continued)

Category	Food	Total folate (μg/100 g) (min–max values when available)
	Lupin, bean	355
	Mungo beans, dried /cooked	421 / 94
	Peas, frozen, raw / cooked	87 / 68
	Red beans, dried /cooked	394 / 78
	Soy, whole seed	328 (280–375)
	Soy flour	573 (345–800)
	Split peas, dried / cooked	154 / 119
	White bean, dried	308 (226–388)
Spices and herbs	Basil (sweet), dried / fresh	310 / 68
	Cayenne pepper	106
	Chervil, fresh	220
	Coriander, fresh	60
	Dill, fresh	116
	"Herbes de Provence"	274
	Laurel, leaf, dried	180
	Marjoram (sweet), dried	274
	Mint, dried /fresh	530 / 110
	Onions, dried	166
	Oregano, dried	237
	Parsley, dried / fresh	187 / 134
	Rosemary, dried / fresh	307 / 109
	Saffron	93
	Sage, dried / fresh	274 / 72
	Sesame seed, raw / husk removed / tahin	97 / 96–106 / 98
	Thyme dried	274
Starchy vegetables	Chestnut, flour / chestnuts, grilled / raw	215 / 70 / 60
	Parsnip, raw / cooked	67
	Potato chips, low fat	254
	Potato starch	82
Vegetables	Artichokes, cooked / raw	89 / 68
	Asparagus, any color, raw / raw, peeled / boiled / preserve	150 / 101 / 142 / 72
	Avocado	80
	Beetroot (red), raw / cooked	100 / 80 / 75
	Broccoli, raw / frozen / cooked	153 (42–275) / 80
	Brussel sprouts, cooked	101–113
	Cabbage, green, raw	68–101
	Cabbage, white, raw	77
	Cardoon, raw	68
	Cauliflower, raw / frozen / cooked	111 / 64 / 79

(continued)

Table 8 (continued)

Category	Food	Total folate (μg/100 g) (min–max values when available)
	Celeriac, raw / cooked	86 / 67
	Corn salad	60
	Leek, raw	73
	Lettuce, raw	136
	Pepper (red), raw	67
	Rocket, raw	97
	Scarole or "Frisée" chicory, raw	126–142
	Spinach, raw / deep frozen / plantlets (raw) / cooked	207 (150–300) / 184 / 128 / 121–125
	Tomato dried / puree	68 / 72
Various	Cocoa powder, no sugar added	107
	Pollen, fresh	993

hydrolysis (Jägerstad and Jastrebova 2013); as described for N5-methyl THF, thermal treatment of food can induce formation of rearrangement or glycation products (Verlinde et al. 2010). N5-formyl THF is a rather stable form but can be chemically converted into the less stable N10-formyl THF (see Fig. 20).

- Their limited bioavailability (Hannon-Fletcher et al. 2004). Although polyglutamoylation may be suspected for that, it seems that it is not usually limiting in the intestinal absorption of folates (McKillop et al. 2006). The decisive reasons would be matrix effect, as folates bind to proteins and sugars, which justifies the use of various treatments for quantifications in food and biological samples (heat, acid, acetone and/or enzymes) (Pfeiffer et al. 2009). Conjugase inhibitory activity may also explain lowered polyglutamate bioavailability in some foods (Bhandari and Gregory 1990).

For these reasons, folic acid, a very minor form of natural vitamin B9 which is fully oxidized on the pyrazine ring, has long been considered the best form of administration: it is a single chemical entity, the simplest form (without one-carbon unit and monoglutamoylated), chemically stable, and used as a synthetic derivative without the risk of matrix effect. The Institute od Medicine (1998) has suggested the use of dietary folate equivalents (DFE), where foof folates are considered to be considerably less bioavailable than folic acid:

μg DFE = μg food folates + μg folic acid × 1,7
1 μg DFE = 1 μg food folate = 0.6 μg folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a folic acid supplement taken on an empty stomach

Table 9 Folate composition in foods rich in folates

Species	Total folate	Folic acid	N10-folate	Formyl THF vitamers	N10-formyl THF	N5,N10-methenyl THF	N5-formyl THF	N5-methylTHF	THF	References
Beans (kidney), dry	115	9	–	60	–	–	–	27	19	Ložnjak et al. (2020)
Blackberry	102	–	–	*19.5*[a]	–	*11*	*8.5*	*73.5*	–	Zou et al. (2019)
Broccoli	130	<0.1	–	9	–	–	–	108	13	Ložnjak et al. (2020)
Cabbage (Pak-choi)	*163.5*	*16.5*	*4.5*	*107.5*[a]	*38.5*	*16.5*	*52.5*	*56.5*	*12*	Shohag et al. (2017)
Cherry	92	–	–	*15*[a]	–	*5*	*10*	*71*	–	Zou et al. (2019)
Leek	109	<0.1	–	2	–	–	–	110	1	Ložnjak et al. (2020)
Lentils	81	2	–	21	–	–	–	36	22	Ložnjak et al. (2020)
Lettuce	*94*	*9*	*5*	*27*[a]	*6*	*13.5*	*7.5*	*59*	*21*	Shohag et al. (2017)
Peanuts	81	7	–	65	–	–	–	7	2	Ložnjak et al. (2020)
Peas (green), frozen	87	1	–	6	–	–	–	75	6	Ložnjak et al. (2020)
Romaine salad	111	<0.1	–	9	–	–	–	101	1	Ložnjak et al. (2020)
Spinach leaves	*225*	*10*	*4*	*65*[a]	*14*	*25*	*26*	*135*	*18*	Shohag et al. (2017)
Strawberry	89	<0.1	–	7	–	–	–	80	2	Ložnjak et al. (2020)
	131	–	–	*5*[a]	–	*2.5*	*2.5*	*113.5*	–	Zou et al. (2019)

[a]Estimation from addition of reported formyl vitamers in charts

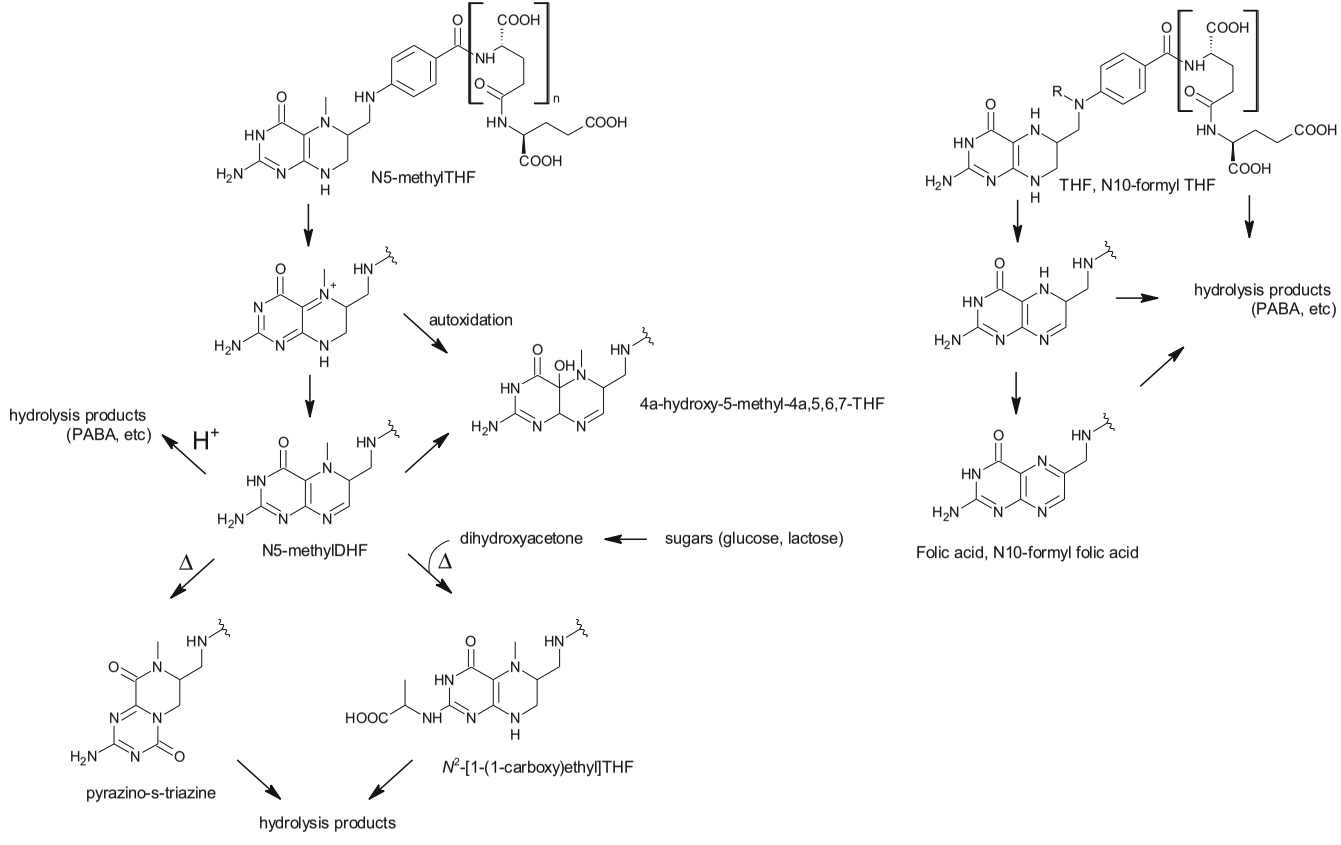


Fig. 23 Degradation reactions of major food tetrahydrofolates in food

Yet, this assumption is still debated, with numerous papers suggesting higher bioavailability for food folates, while the effectiveness of food fortification with folic acid, which is mandatory in some countries and has been described as "highly effective in decreasing NTD cases in populations where it has been implemented" (Caffrey et al. 2018), has recently been more cautiously addressed in a meta-analysis (Centeno Tablante et al. 2019).

As usually reported for water-soluble vitamins, folate loss by plain extraction should not be overseen. It is possibly important in industrial processes (Delchier et al. 2013) as well a cooking methods (Leichter et al. 1978) and can involve any natural or synthetic folate form.

26.3.6 Safety: Toxicity and Side Effects

Direct toxicity of folates is considered to be negligible within normal range of consumption, but may be lethal at very high folic acid doses (Devnath et al. 2017). A specific risk must be considered in the context of vitamin B12 deficiency, as folate can correct megaloblastic anemia and make it difficult to identify. High dose folic acid may be correlated with an increased detection of some (prostate) cancers, while it clearly prevents others (e.g., colorectal cancer) (Patel and Sobczyńska-Malefora 2017). This may be explained by a stimulation of cell proliferation in cancer tissue. It is not clear whether any of the above applies to natural folates.

26.3.7 Marketed Products

The predominant form used for vitamin B9 supplementation is folic acid. N5-methylTHF has been suggested as an alternative, because it does not require MTHFR processing (MTHFR polymorphism is the most frequent cause for enhanced folate requirement in the general population) (Guéant-Rodriguez et al. 2006) and cannot mask vitamin B12 deficiency (it depends on cobalamin for conversion into methylene THF, thus does not apparently corrects the effects of cobalamin deficiency as folic acid does) (Scaglione and Panzavolta 2014). Foods can be promoted on the basis of their amount in folates and possible health benefits in Europe (see Sects. 3.3 and 3.4). Recently, natural extracts have been marketed as natural vitamin B9.

Inhibitors of specific enzymes in folate metabolism have been developed and used as antimicrobials and anticancer agents. Their use is frequently associated with supplementation in N5-formyl THF in order to enhance their activity or lower their toxicity to humans. They are summed up in Table 10.

26.3.8 Patents

Enhanced bioavailability is a subject of interest for folate. A registered glucosamine salt folate salt has been reported to have grater bioavailability than folic acid in rats (Miraglia et al. 2016).

Table 10 Drugs affecting folate metabolism

Compound	Target (effect)	Intended use	Associated folate form and aim
5-fluoro uracile (5FU, fluorouracil)	Human thymidylate synthase inhibitor	Cancer chemotherapy	N5-formyl THF (folinic acid), enzyme cofactor enabling suicide inhibition
Methotrexate (MTX, amethopterin)	Human dihydrofolate reductase inhibitor (folate analogue)	Cancer chemotherapy, immunodepressant	N5-formyl THF (folinic acid), diminishes MTX toxicity – not affected by DHFR inhibition
Pyrimethamine	Parasite dihydrofolate reductase inhibitor	Toxoplasmosis treatment	N5-formyl THF (folinic acid), diminishes MTX toxicity – not affected by DHFR inhibition
Sulfamethoxazole	Dihydropteroate synthase inhibitor in bacteria	Antibacterial (synergism with trimethoprime)	-
Trimethoprime	Bacterial dihydrofolate reductase inhibitor	Antibacterial (synergism with sulfamethoxazole)	N5-formyl THF (folinic acid), diminishes MTX toxicity – not affected by DHFR inhibition

26.3.9 Perspectives

While the effects of defects in folate status or metabolism are clearly established, choices for supplementation are still debatable. Difficulties in analysis, links between specific ingested forms, biomarkers, and health effects explain the need for further work and standardization in assessment methods.

26.4 Vitamin C: Ascorbic Acid

26.4.1 Bioactive Constituents

Ascorbic acid, also known as vitamin C, is a 6-carbon hydroxylactone biogenetically derived from glucose with a molecular mass of 176.12 Da. It is a water-soluble vitamin, which is sensitive to heat and light (EFSA NDA Panel 2013b). It was first isolated by Albert Szent-Gyorgyi in 1928 and identified by Norman Haworth in 1933 (Carpenter 2012; Padayatty and Levine 2016). It has two pKa, with the first at 4.2 and the second at 11.6. Thus, at pH = 7, vitamin C is mostly present in its ionized form, ascorbate (99.9%) (Du et al. 2012). This molecule is often in the form of salts, among them sodium ascorbate and calcium ascorbate are the most common. A resonance form exists for this deprotonated anionic form in particular due to the presence of the two conjugated double bonds (Linster and Van Schaftingen 2007). Ascorbic acid, the functional in vivo form of the vitamin, is the enolic form of an α-ketolactone with an asymmetric carbon atom. Only one of its stereoisomers is sufficiently active, *L*-(+)-ascorbic acid. The two enolic hydrogen atoms confer the

acidic character to the molecule and provide electrons for its function as antioxidant. In solution, vitamin C can be oxidized and give a mixture of ascorbate and dehydroascorbate (EFSA NDA Panel 2013b; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Linster and Van Schaftingen 2007; Smirnoff 2018) (Fig. 24).

The first oxidation step of vitamin C is the loss of one electron to provide the derived resonance-stabilized MDHA (monodehydroascorbate) radical, which is central to its biological activities. Its half-life is longer (many seconds or even minutes) than many others radical species, depending on absence of oxygen and electron acceptors such as iron. If many redox reactions are common in plants and mammals, this step can be managed by specific enzymes in plants, in particular ascorbate peroxidase (APX) and ascorbate oxidase (AO). DHA (dehydroascorbate) and ascorbate are then produced by dismutation of MDHA with an equilibrium in favor of ascorbate and DHA. The tri-carbonyl structure of DHA is particularly instable and is present in the hydrated hemiacetal form in solution (Du et al. 2012; Linster and Van Schaftingen 2007; Smirnoff 2018) (Fig. 24). Hence, vitamin C is a good free radical scavenger in vitro and in vivo by both its ability to act as an electron donor with a redox potential and the relative stability and unreactivity of MDHA (Paciolla et al. 2019). By its capacity to donate reducing equivalents, vitamin C also contributes to several biochemical reactions. It is a cofactor for several enzymes involved in the human metabolism, in particular Cu^{+}-dependent

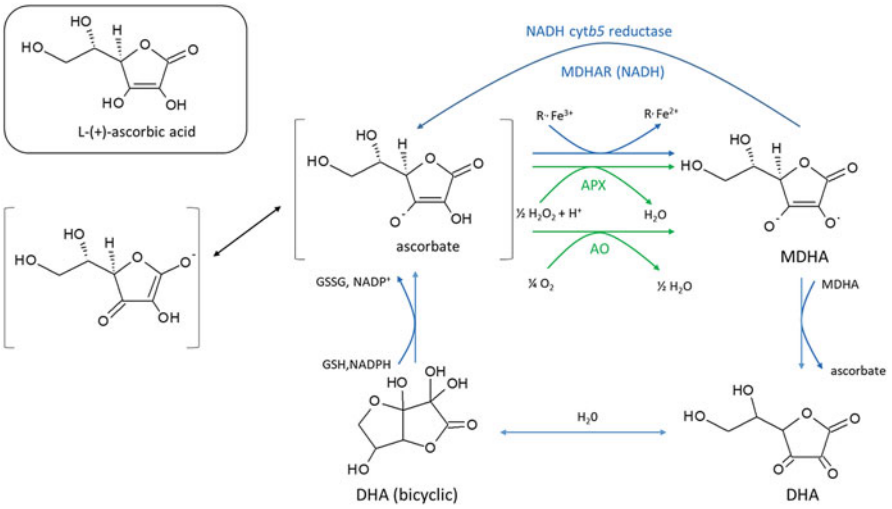


Fig. 24 Structure of L-(+)-ascorbic acid and its redox states (Adapted from (Linster and Van Schaftingen 2007; Smirnoff 2018). Ascorbate, fully reduced form; MDHA, monodehydroascorbate, resonance-stabilized ascorbate radical; DHA, dehydroascorbate, fully oxidized form. Reactions occurring both in plants and mammals are shown in blue and plant specific reaction are shown in green. In plants, MDHA and DHA reduction are catalyzed respectively by NADH- and GSH-dependent enzymes families and in mammals by a variety of enzymes

monooxygenases and Fe^{2+}-dependent dioxygenases. It is particularly involved in the biosynthesis of collagen, carnitine, and some neurotransmitters. It also contributes to iron absorption, immune stimulation, and epigenetic regulation (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Paciolla et al. 2019).

Vitamin C is an essential nutrient involved in the normal metabolism of humans and many other mammals. If some animals are able to synthetize it from D-glucuronate, derived from UDP-glucuronate, in some organs such liver or kidney, others including humans, nonhuman primates, guinea pigs, or bats have lost this capacity. They no longer have the last enzyme of the pathway, L-guluno-1,4-lactone oxidase, due to an accumulation of mutations in its coding gene. No evolutionary explanation is satisfactory yet (Fenech et al. 2019; Linster and Van Schaftingen 2007).

Plant-derived ascorbate is the major source of vitamin C in the human diet. Indeed, L-ascorbate is abundant in plants, but with contents significantly different among plant species and cultivars (Paciolla et al. 2019; Smirnoff 2018). Ascorbate concentrations vary between the organs, with highest contents in the aerial parts such as leaves and flowers and lower contents in less photosynthetically organs such as roots and stems. Ascorbate concentrations can reach 1–5 mM in leaves. In seeds, ascorbate accumulates during early germination and disappears at maturity and when they are deshydrated. Vitamin C occurs in all cell compartments, with concentrations up to 20 mM in chloroplasts. It is also found in the apoplast (cell wall and extracellular space) but with lower contents and in a more oxidized form than in the intracellular pool. Different environmental changes, and in particular light, can impact the regulation of vitamin C levels (Paciolla et al. 2019; Smirnoff 2018; Smirnoff and Wheeler 2000; Wheeler et al. 1998).

L-ascorbate has several roles in plant (Fenech et al. 2019; Paciolla et al. 2019; Smirnoff 2018):

- It contributes to the smooth running of photosynthesis by different ways and slows down leaf senescence: as a ROS scavenger limiting ROS-mediated damage; as an enzyme cofactor, in particular as a cofactor of violaxanthin de-epoxidase, an enzyme involved in preventing photodamage, by converting violaxanthin to zeaxanthin, thus dissipating excess excitation energy; as donate electrons when photosystems are damaged by stress conditions.
- It is involved in the synthesis of plant hormones as an enzyme cofactor.
- It is implicated in the control of plant growth and development, as well as in the plant defense response against pathogens.
- It is involved in transmembrane electron transport and it favors iron uptake and transport in plants.
- It is particularly involved in the control of cell division; the ascorbate redox state has a major role in the cell cycle progression.
- It also plays a role in the programmed cell death induced among others by H_2O_2 formation of heat shock. It is to note that APX, an enzyme family limited to green plants and photosynthetic protists, contributes to the high capacity of plants to

remove H_2O_2 produced by photosynthetic electron transport and photorespiration, by converting it to H_2O and O_2.

The biosynthesis of ascorbate in higher plants is subject to discussion by different researchers. Unlike the animal pathway, in higher plants, no carbon conversion is observed. The carbon 1 in the D-glucose remains as the carbon 1 in ascorbate after conversion.

The predominant pathway of vitamin C biosynthesis seems to be that proposed by Smirnoff and Wheeler in 1998 (Wheeler et al. 1998). The **Smirnoff-Wheeler pathway** (**D-mannose/L-galactose pathway**) involves D-mannose and L-galactose (Fig. 25). The two initial steps begin by the conversion of D-glucose-6-phosphate into D-mannose-6-phosphate, which is then transformed into D-mannose-1-phosphate by the action of a phosphomannose mutase (PMM). GDP-D-mannose is formed from D-mannose-1-phosphate by the transfer of guanosine monophosphate from GTP by GDP-D-mannose-pyrophosphorylase (GMP). GDP-L-galactose is further produced from GDP-D-mannose by a GDP-D-mannose epimerase (GME) through a 3′,5′-epimerization. In the Smirnoff-Wheeler pathway, GDP-L-galactose is converted into L-galactono-1,4-lactone in several steps in the cytosol. Several enzymes are implicated: GDP-L-galactose-phosphorylase (GGP), L-galactose-1-phosphate phosphatase (GPP), and L-galactose dehydrogenase (LGal-DH). L-galactono-1,4-lactone must then move from cytosol to mitochondria where the L-galactono-1,4-lactone dehydrogenase is localized and allows the conversion into ascorbate transferring electrons to cytochrome c. It is interesting to note that this last step of oxidation is managed by a deshydrogenase instead of an oxidase as occurs in animals (L-guluno-1,4-lactone oxidase). This deshydrogenase allows not to release H_2O_2 and does not affect the redox state of the cell during the formation of ascorbate (Fenech et al. 2019; Paciolla et al. 2019).

The other ascorbate biosynthetic pathways are (Fig. 25):

- The **gulose pathway** (**Wolucka-Van-Montagu pathway**) starts from the 5′-epimerization of the GDP-D-mannose with the formation of GDP-L-gulose. Indeed, GME is also able to catalyze the 5′-epimerization instead of 3′,5′-epimerization described in the Smirnoff-Wheeler pathway (Major et al. 2005). GDP-L-gulose could be converted into L-guluno-1,4-lactone in several steps, but some enzymes are not clearly identified. Then, the gulunolactone oxidase (GulLo) could be transformed L-guluno-1,4-lactone into ascorbate (Paciolla et al. 2019). Other authors hypothesize that ascorbate could be directly synthetized from GDP-L-gulose, due to its scarcity in plants and its lack of structural function (Fenech et al. 2019).
- The **myoinositol pathway**, following a pathway similar to animals, could also lead to the formation of L-guluno-1,4-lactone. First, myoinositol is oxidized in D-glucuronate by the myoinositol oxygenase (MIOX). Then, D-glucuronate is converted into L-guluno-1,4-lactone in two steps by glucoronate reductase and aldono lactonase (Paciolla et al. 2019; Valpuesta and Botella 2004). However, the contribution of the myoinositol pathway in the ascorbate biosynthesis remains still unclear (Fenech et al. 2019).

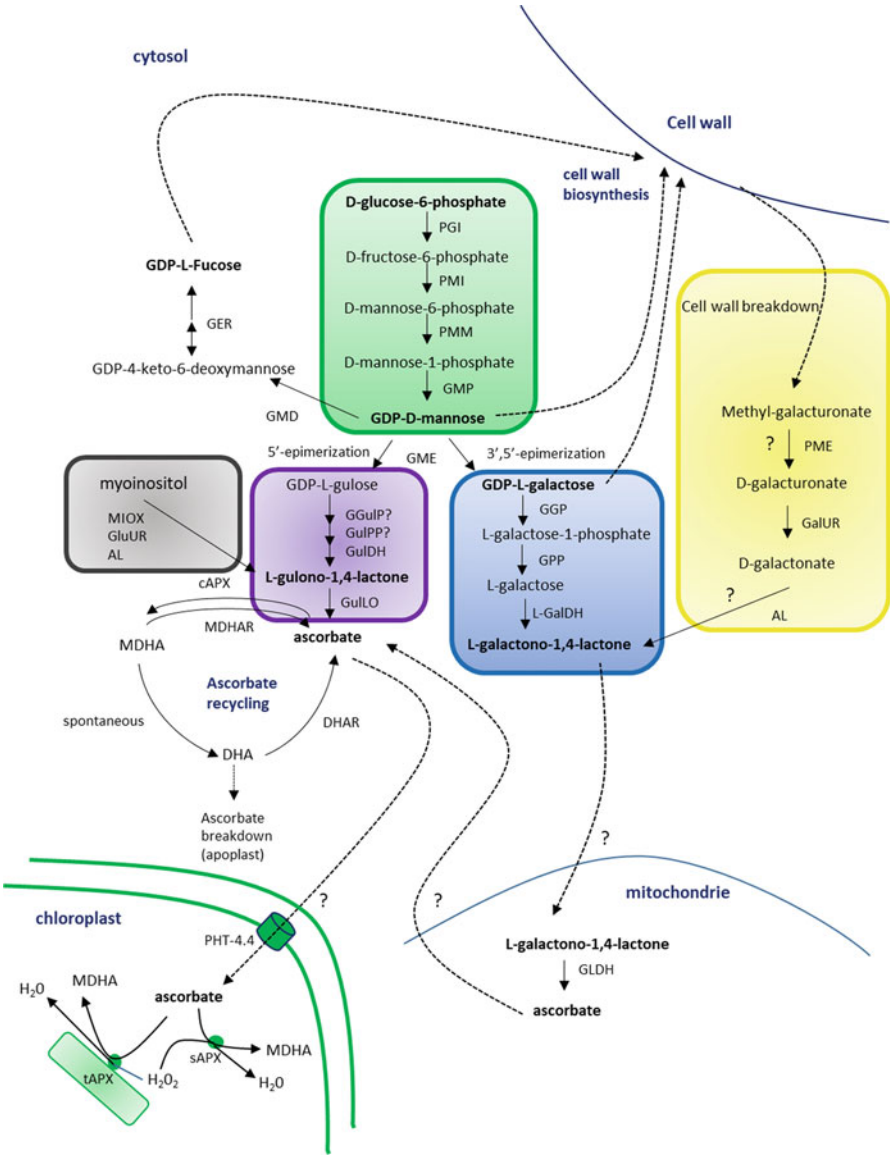


Fig. 25 Biosynthetic pathways of ascorbate in the plant cells (Adapted from Fenech et al. 2019; Paciolla et al. 2019). Different colors indicate different pathways. In gray, the myo-inositol pathway; in violet, the gulose pathway (Wolucka-Van-Montagu pathway); in blue, the D-mannose/L-galactose pathway (Smirnoff-Wheeler pathway); in yellow, the galacturonate pathway. In green are represented the initial steps leading to GDP-D-mannose. Solid lines represent the dedicated reactions within a pathway. Dashed lines represent the translocation of a molecule from a cellular compartment to another. A question mark indicates enzymes not identified in plants. Abbreviations of enzymes: AL, aldono reductase; cAPX, cytosolic ascorbate peroxidase; DHAR, dehydroascorbate reductase; GalUR, D-galacturonate reductase; GGP, GDP-L-galactose-phosphorylase; GGulP, GDP-L-gulose pyrophosphatase; GLDH, L-galactono-1,4-lactone dehydrogenase;

- The **D-galactorunate pathway**, mainly active during fruit ripening in some species, uses methyl-galacturonate released by the degradation of pectin during the cell wall breakdown. Methyl-galacturonate is converted into D-galacturonate by a pectin methyl esterase. D-galacturonate is then used by a D-galacturonate reductase (GalUR) to produce D-galactonate that is converted by an aldone-lactonase into L-galactono-1,4-lactone, the last intermediate within the Smirnoff-Wheeler pathway (Agius et al. 2003; Fenech et al. 2019; Paciolla et al. 2019).

In addition to its biosynthesis, the ascorbate pool can also come from its recycling (Foyer-Halliwell-Asada cycle) and depend on its degradation (Fenech et al. 2019). Moreover, several ascorbate derivatives, including glucosides, have been identified in plants (Smirnoff 2018).

26.4.2 Bioavailability and Metabolism

26.4.2.1 Bioavailability

Absorption

Oral route of administration is privileged for vitamin C that comes from food, mainly fruits and vegetable, or supplements. In general, typical human diet provides sufficient amount of vitamin C in healthy population. However, this dietary intake can be inadequate in the case of some pathologies or in smokers (Lykkesfeldt and Tveden-Nyborg 2019). Absorption of ascorbate and DHA mainly occurs in the enterocytes of the small intestine. Three modes of membrane transport are possible: active transport, passive, and facilitated diffusion. At low gastrointestinal concentrations, ascorbate is absorbed through specific sodium-dependent active transporters belonging to nucleobase transporters and named SVCTs (Sodium-Dependent Vitamin C Transporters). The high capacity/low affinity transporter of vitamin C, SVCT1, is particularly implicated. This transport process in intestinal epithelium is saturable and highly dose-dependent. On the other hand, its release into the blood stream is less elucidated. DHA is accumulated in cells through facilitated diffusion via sodium-independent facilitative glucose transporter (GLUTs), in particular GLUT 1 and GLUT3. Once inside the cell, DHA van be converted to ascorbate by

Fig. 25 (continued) GluUR, glucoronate reductase; GMD, GDP-D-mannose-4,6-dehydratase; GER, GDP-4-keto-6-deoxymannose,3,5-epimerase-4-reductase; GME, GDP-D-mannose epimerase; GMP, GDP-D-mannose-pyrophosphorylase; GPP, L-galactose-1-phosphate phosphatase; GulDH, L-gulose dehydrogenase; GulPP, L-gulose-1 phosphate phosphatase; GulLO, gulonolactone oxidase; L-GalDH, L-galactose dehydrogenase; MDHAR, monodehydroascorbate reductase; MIOX, myoinositol oxygensase; PGI, phosphoglucose isomerase; PME, pectin methyl esterase; PMI, phosphomannose isomerase; PMM, phosphomannose mutase; sAPX, stromal ascorbate peroxidase; tAPX, thylakoidal ascorbate peroxidase. Abbreviations of substrates: DHA, dehydroascorbate; MDHA, monodehydroascorbate.

intracellular reduction or transported to the blood stream by GLUT1 and GLUT2. Finally, a passive diffusion could play a potential role in its absorption, in particular at high concentrations. Indeed, at pH 5 in the small intestine, the proportion of unionized ascorbic acid goes from 0.1% to 15% (Du et al. 2012; Lykkesfeldt and Tveden-Nyborg 2019; Padayatty and Levine 2016). Eighty percent of ascorbic acid is absorbed for a dietary intake of 100 mg/day and about 75% for an intake of 1g/day; however, efficiency of absorption gradually decreases at higher intakes and falls to about 50% or less with doses above 1 g/day. The bioavailability of vitamin C from foods and supplements does not seem to be significantly different (EFSA NDA Panel 2013b; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000).

After absorption, vitamin C is transported in plasma predominately in the free reduced form as ascorbate. No specific binding protein is known (EFSA NDA Panel 2013b). The peak plasma of vitamin C concentrations is reached 120–180 min after ingestion (Padayatty and Levine 2016). Negligible amount of oxidized vitamin C is present in plasma (Lykkesfeldt and Tveden-Nyborg 2019). A difference can be noted between women and men, because women reach the plateau of plasma ascorbate concentration at lower vitamin C intake. Moreover, the plasma ascorbate concentration decreases in pregnant women. Smokers have lower plasma ascorbate concentration than non-smokers (EFSA NDA Panel 2013b). In humans, plasma concentrations are firmly controlled when ascorbate is taken orally (Graumlich et al. 1997; Levine et al. 1996). The normal range for ascorbate in blood plasma is 40–80 μmol/L. Due to homeostatic regulation, steady-state plasma concentrations do not exceed 220 μmol/L even if the maximum tolerated oral dose is taken (3 g 6 times daily). By contrast, when ascorbate is administered parenterally, for a 50 g intravenous dose, plasma concentrations can reach 15,000 μmol/L (Padayatty et al. 2004). At these extracellular concentrations, vitamin C could be toxic for cancer cells, hence clinical research studies are conducted on the use of vitamin C by the IV route in the supportive care of cancer patients (Du et al. 2012; Fritz et al. 2014; Klimant et al. 2018; Ngo et al. 2019).

Distribution

Vitamin C is distributed from blood stream to all tissues. This distribution is highly compartmentalized. The cellular transport of ascorbate and DHA is mediated by active and facilitative transport systems respectively. SVCT1 and more specifically SVCT2 contribute to the cellular transport of ascorbate in various tissues and maintain intracellular levels of ascorbate under normal physiologic conditions. These transporters have different tissue distribution and vary by cell type. The low capacity/high affinity of vitamin C transporter, SVCT2, is expressed in a lot of tissues (EFSA NDA Panel 2013b; Lykkesfeldt and Tveden-Nyborg 2019). The cellular transport of DHA is mediated by facilitative transport by means of glucose transporters, in particular GLUT1, GLUT 3, and GLUT 4. In some cell types, the uptake of DHA is inhibited by physiological concentrations of glucose. To maintain the ascorbate pool, DHA is efficiently converted into ascorbate intracellularly by several cell types (Du et al. 2012; EFSA NDA Panel 2013b). In healthy individuals,

intracellular concentrations of ascorbate are higher than extracellular fluids. Tissue concentrations are in the range of mM, while they are in the range of μM in plasma. These concentrations are higher than those necessary for some biological activities of vitamin C, such as its actions as coenzyme, suggesting that vitamin C could have other yet unknown functions. Only erythrocytes have no SVCT transporters and have ascorbate level similar to that of plasma. Human erythrocytes have a recycling capacity of ascorbate from DHA (Du et al. 2012; Lykkesfeldt and Tveden-Nyborg 2019; Padayatty and Levine 2016). Adrenal glands and pituitary glands reveal the highest ascorbate concentrations. Liver and brain also show high concentrations (Du et al. 2012; Padayatty and Levine 2016).

26.4.2.2 Metabolism

In the body, the metabolism of ascorbate is closely related to its biological roles as free radical quencher and as cofactor, leading to its oxidation to DHA. Since DHA is readily reduced in ascorbate intracellularly by a number of cell types to maintain the ascorbate pool, relatively small amounts of the vitamin are lost during the catabolism. A part of DHA is irreversibly hydrolyzed to 2,3-diketogulonic acid, which can then be decarboxylated to L-xylonic acid and L-lyxonic acid. In some animals, these metabolites may enter in the pentose phosphate pathway (Lykkesfeldt and Tveden-Nyborg 2019). Other metabolites products have been identified as L-xylose, L-threonic acid, and oxalic acid. In humans, oxalic acid is the main metabolite produced from 2,3-diketogulonic acid and clinically identified in the urine. Indeed, urine is the main route of elimination of unchanged ascorbate and its metabolites. In the kidneys, due to its hydrophilic nature and low molecular weight, ascorbate is filtered by glomerulus through a hydrostatic pressure gradient and concentrated in the urine. Due to the formation of oxalic acid from DHA, in particular with high doses of vitamin C, its consumption could lead to calcium oxalate accumulation in the urinary space in individuals with impaired renal function (EFSA NDA Panel 2013b; Levine et al. 1996; Padayatty and Levine 2016). Likewise, with large intakes of vitamin C, unabsorbed ascorbate is degraded by the intestinal microflora, which may cause diarrhea and intestinal discomfort sometimes reported by consumers of large doses of vitamin C (EFSA NDA Panel 2013b; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000).

In addition to dose-dependent absorption, a renal reuptake of ascorbate contributes to the regulation of body ascorbate content. This renal reuptake, highly concentration-dependent, takes place in the proximal renal tubules mainly by means of saturable active transport through SVCT1 transporters. Ascorbate could then release in the blood stream by a poorly understood diffusion mechanism. Passive reabsorption of vitamin C does not seem to play an important role. DHA formed from ascorbate in the renal epithelium is released in the blood stream by GLUT2 transporters located in the basolateral membrane. Under saturated conditions, ascorbate will be preferentially excreted, whereas at very low ascorbate intakes, no ascorbate will be excreted to limit the loss (EFSA NDA Panel 2013b; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Lykkesfeldt and Tveden-Nyborg 2019).

Vitamin C is secreted in breast milk and the mean of vitamin C concentration in human milk from lactating women without vitamin C supplementation is in the range of 35–50 mg/L (EFSA NDA Panel 2013b).

26.4.3 Bioactivities

26.4.3.1 General Role

The two main functions of vitamin C are:

- To be a cofactor of enzymatic reactions
- To be a reducing and antioxidant agent

Vitamin C as a Cofactor of Enzymatic Reactions

By its capacity to donate reducing equivalents, vitamin C is an enzyme cofactor for several biochemical reactions mainly catalyzed by monooxygenases and dioxygenases, including those in which it is an actual cosubstrate. In mammals, vitamin C is the cofactor of more than 15 dioxygenases and at least three monooxygenases. These enzymes are the more often copper or iron-dependent proteins. Vitamin C reduces the active center metal ion of these enzymes and maintains the formed metal ions in a reduced state for their optimal activity (EFSA NDA Panel 2013b; Padayatty and Levine 2016):

- Vitamin C is particularly important for the biosynthesis of collagen, the most abundant protein in mammals, which represents approximately 25% of the total protein content. Collagen is the major component of the extracellular matrix that supports most tissues in particular connective tissues. This protein is found in skin, cartilage, ligaments, tendons, bone, and teeth. Its amino acid composition is atypical for proteins due to its high hydroxyproline content. Vitamin C is particularly implicated in the hydroxylation of the amino acids proline and lysine of the procollagen, a crucial step for later glycosylation and the formation of the triple helix structure of collagen. Vitamin C acts as a cofactor of three dioxygenases implicated in procollagen hydroxylation: procollagen-proline dioxygenase (three isoenzymes of **the collagen prolyl 4-hydroxylase** and **collagen prolyl 3-hydroxyase**) and procollagen-lysine 5-dioxygenase (**lysyl hydroxylase)**. A deficiency in vitamin C for a long period results leads to impaired collagen synthesis and scurvy (Du et al. 2012; Pullar et al. 2017).
- Vitamin C also contributes to the regulation of the activity of the hypoxia-inductible factors (HIFs), in particular HIF-1. Ascorbate is the cofactor of three isoenzymes of the **HIF prolyl 4-hydroxylase**, which regulate the activity of HIFs by the hydroxylation of specific proline residues on HIF-α, the regulatory oxygen-sensitive subunit of the heterodimeric HIF. Under normal conditions, HIF-1α is hydroxylated and degraded by proteasomes leading to the decrease of HIFs activity, whereas under hypoxia conditions or ascorbate depletion, the hydroxylation is inhibited, HIF-1α is stabilized, and forms a heterodimer with the oxygen-

insensitive subunit HIF-β leading to the activation of HIF activity. HIF-1 is also negatively regulated by FIH-1 (factor inhibiting HIF) or **asparaginyl hydroxylase** which also requires vitamin C as cofactor. So, ascorbate availability will mainly have an impact of immune cell function in hypoxic inflammatory and tumor surroundings by the regulation of HIFs activation. Because of more than 60 gene products are regulated by HIF, ascorbate availability may also have an significant impact on several cell functions among others erythropoiesis, angiogenesis, cell proliferation and survival, glucose and iron metabolism (Ang et al. 2018; Padayatty and Levine 2016).

- Vitamin C is also a cofactor for the **lysine-specific demethylase 1** (LSD1), a JmjC domain-containing histone demethylase belonging to the superfamily of the flavin adenine dinucleotide (FAD)-dependent amine oxidases. LSD1 is a Fe^{2+}-2-oxoglutarate dioxygenase. This enzyme is able to demethylate trimethylated lysines of histone via a redox process. LSD1 could play a significant role in different biological processes as cell proliferation or tumor progression. Ascorbate is also a cofactor for **ten-eleven translocation (TET) methylcytosine dioxygenases** that catalyze DNA demethylation. Ascorbate availability can influence the demethylation of histone and DNA. Due to the correlation of DNA hypermethylation and cancer development, as well as the impact of TET DNA demethylases and some histone demethylases on epigenetic remodeling of immune cells, ascorbate could have an impact in immune cell function and prevention of cancer (Ang et al. 2018; Camarena and Wang 2016; Chen et al. 2012; Du et al. 2012).
- Vitamin C plays a role, at least in vitro, in the biosynthesis of carnitine, a quaternary ammonium compound implicated in energy catabolism. It mainly contributes to the transport of fatty acids in mitochondria for ATP formation. Vitamin C is as a cosubstrate of two dioxygenases, **trimethyllysine hydroxylase** and **γ-butyrobetaine hydroxylase**. The role of vitamin C in the biosynthesis of carnitine in-vivo is more difficult to characterize (Dunn et al. 1984; Rebouche 1991a, 1991b, 1995).
- Vitamin C is a cosubstrate for the 4-hydroxyphenylpyruvate dioxygenase, a Fe (II)-containing non-heme oxygenase that catalyzes the second reaction in the catabolism of tyrosine (Moran 2005).
- Vitamin C is a cosubstrate for catecholamine biosynthesis, in particular the conversion of dopamine to noradrenaline in nervous system and in the adrenal glands. This conversion implicates the monooxygenase known as **dopamine β-hydroxylase** (Menniti et al. 1986).
- The activity of another monooxygenase, the **peptidylglycine α-amidating monooxygenase**, located in secretory vesicles, is also known to be dependent on vitamin C and also other electron donors. This enzyme catalyzed the first step of peptide amidation that is essential for the activity of several hypothalamic and gastrointestinal hormones such as oxytocin, vasopressin, cholecystokinin, and α-melanocyte-stimulating hormone (Kolhekar et al. 1997; Prigge et al. 1999, 2000).
- Vitamin C is implicated in the transformation of cholesterol to bile acids via the enzyme **cholesterol 7α-monooxygenase**, known also as cholesterol 7α-

hydroxylase. Reduced cholesterol conversion to bile acids induces cholesterol accumulation in the liver and blood and atherosclerotic modifications in coronary arteries (EFSA NDA Panel 2013b; Ginter 1989; Turley et al. 1976). Vitamin C also contributes to the regulation of steroid metabolism and steroidogenesis in the adrenal glands. These reactions require the microsomal enzymatic system containing cytochrome P-450 hydroxylase. It is also to note that reducing agents such as vitamin C may enhance the hydroxylation of aromatic drugs and carcinogens by hepatic cytochrome P-450 (EFSA NDA Panel 2013b).

These many enzymatic functions have been established in vitro but not usually in vivo (Padayatty and Levine 2016).

Vitamin C as a Reducing and Antioxidant Agent

Apart from its implication in several biochemical reactions as a cofactor, vitamin C is well-known to be a reducing agent or electron donor and may exert beneficial activities as a water-soluble antioxidant agent. As an electron donor with redox potential and by the relative stability and unreactivity of the ascorbate radical MDHA, vitamin C is a good free radical scavenger both in vitro and in vivo of reactive oxygen species and reactive nitrogen species, as well as singlet oxygen and hypochlorite. The one- or two-electron oxidation products, MDHA and DHA, are relatively nontoxic and are easily regenerated in vivo. This chemical and enzymatic regeneration goes through glutathione and NADH or NADPH reductases (Fig. 24).

Vitamin C could be part of the antioxidant defense system directly. It could for example protect the eye against oxidative damage, in particular against photoinduced free radicals, or the neurophils against ROS produced during phagocytosis. Vitamin C also inhibits the low-density lipoprotein (LDL) oxidation by scavenging ROS before the initiation of lipid peroxidation, reducing atherosclerosis (Garland 1991' Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Jialal and Devaraj 1996; Moser and Chun 2016). Vitamin C is also responsible for antioxidant protection indirectly by the regeneration of other biological antioxidants such as glutathione and β-carotene from their respective one-electron oxidation products. It is able to recycle vitamin E by reducing α-tocopheroxyl radicals produced via scavenging of lipid radicals (Edge and Truscott 1997; EFSA NDA Panel 2013b; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Kagan et al. 1992).

Vitamin C can also improve dietary non-heme iron gastrointestinal absorption (Bendich and Cohen 1990; EFSA NDA Panel 2013b).

Vitamin C is commonly called as antioxidant, but this term seems to be misleading. Indeed, if some antioxidant functions have been demonstrated in vitro or in some cases in vivo, they are unproven in humans. Supplementation with vitamin C does not seem to display benefits in diseases caused by oxidative stress (Padayatty and Levine 2016).

Moreover, vitamin C could also exert a prooxidant effect in the presence of catalytic metals by reducing ferric to ferrous iron and by releasing ferrous iron from ferritin, which can lead to the formation of free radicals through the Fenton

reaction. However, the link between these effects and an excess of vitamin C intake is not clearly established (Du et al. 2012; EFSA NDA Panel 2013b).

Based on its different biological roles and on different symptoms linked to vitamin C deficiency, European Food Safety Authority (EFSA) authorized several health claims for food and food supplements containing at least 12 mg of vitamin C per 100 g, including (EFSA NDA Panel 2009d, 2010d):

- Vitamin C contributes to the protection of cell constituents from oxidative damage.
- Vitamin C contributes to normal collagen formation and the normal function of bones, teeth, cartilage, gums, skin, and blood vessels.
- Vitamin C contributes to the normal function of the nervous system.
- Vitamin C contributes to the normal function of the immune system.
- Vitamin C increases non-heme iron absorption.
- Vitamin C contributes to normal physiological functions.
- Vitamin C contributes to normal energy-yielding metabolism.
- Vitamin C can contribute to the reduction of tiredness and fatigue.
- Vitamin C contributes to the regeneration of the reduced form of vitamin E.

Moreover, EFSA authorized the claim "Vitamin C contributes to maintain the normal function of the immune system during and after intense physical exercise" only for food which provides a daily intake of 200 mg in addition to the recommended daily intake of vitamin C.

The health claims "Vitamin C contributes to the normal function of the immune system" and "Vitamin C contributes to the protection of cell constituents from oxidative damage" were also authorized for infants and young children from birth to 3 years of age (EFSA NDA Panel 2015b, 2017a).

26.4.3.2 Animal Studies

Like plants, the vast majority of animals are able to synthetize vitamin C through successive reactions which convert D-glucose-1-phosphate to L-ascorbic acid (Fig. 26). This biosynthesis of ascorbate takes place in the liver in mammals, while it is produced in the kidney in fish, amphibians, and reptiles. An inversion of configuration is observed during the biosynthesis due to the formation of a hydroxymethyl group in L-gulonate from the aldehyde function of D-glucuronate. The last step of the biosynthesis leads to the release of H_2O_2, which is not the case in plants. As discussed above, only humans and some animals, among other primates, guinea pigs, bats, and some birds, have lost the ability to synthetize vitamin C due to the highly mutation of L-gulonolactone oxidase that catalyzes the last step in the biosynthesis (Linster and Van Schaftingen 2007; Smirnoff 2018).

A lot of in vivo studies were performed to evaluate the biological activities and bioavailability of vitamin C. Different animal models were employed. For example, rabbits were formerly used to study the effect of ascorbic acid in atherosclerosis, whereas guinea pig was used to elucidate the role of ascorbic acid in the regulation of cholesterol metabolism. The development of model of chronic latent scurvy in the

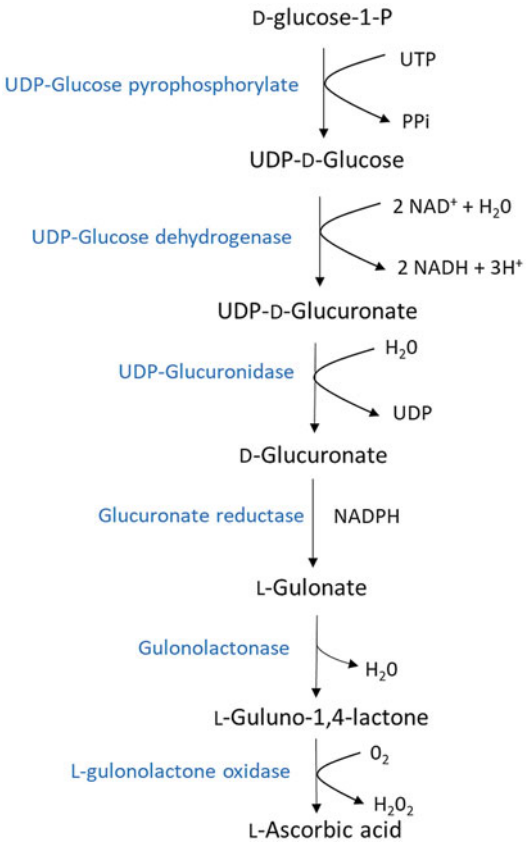


Fig. 26 Biosynthetic pathway of ascorbate in animals. (Adapted from (Linster and Van Schaftingen 2007; Smirnoff 2018))

guinea pig in the 1970s led to significant scientific advances in this domain (Turley et al. 1976). The naturally vitamin C-deficient guinea pig is the model of choice to bioavailability studies. But, genetically scorbutic animal models, such as the Osteogenic Disorder Shionogi (ODS) rats, the L-gulono-γ-lactone oxidase (Gulo−/−) knockout mouse, and the spontaneous bone fracture (sfx) mouse, were developed (Carr and Vissers 2013; Maeda et al. 2000; Mizushima et al. 1984; Mohan et al. 2005). More recent models such as SMP30 (Senescence Marker Protein 30) knockout mice are also employed. These mice develop symptoms of scurvy when subjected to a vitamin deficient diet (Ishigami 2010; Son et al. 2018).

26.4.4 Benefits (Human Studies)

The role of vitamin C in Humans was studied at least since the eighteenth century with the spread of **scurvy** among sailors during the age of exploration. However, this pathology was already known in antiquity. Scurvy is the consequence of a vitamin

Fig. 27 Painting showing some sailors infected by scurvy

C-depleted diet, but the symptoms appear after approximately 1 month of privation. It was precisely the case of long sea travels with a prolonged lack of fresh vegetables and fruits in food diets (Padayatty and Levine 2016). Two million sailors would have been affected by this disease. In 1747, James Lind conducted the first "clinical trial" on several sailors and showed that Citrus were effective in the treatment of scurvy (Fig. 27). At present, this disease is rare but continues to exist, in particular in people affected by malnutrition. It also occurs in adults and children with mental disorders, people with bad eating habits or alcoholism, and older people without medical supervision (Agarwal et al. 2015; Fiorini et al. 2018; Kothari et al. 2020; Marik and Liggett 2019; Urueña-Palacio et al. 2018). Scurvy symptoms can be linked to a total body pool of less than 300 mg and a plasma ascorbate concentration below 10 μM (EFSA NDA Panel 2013b; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000). However, physical signs could only appear at lower plasma ascorbate concentration, as low as 3 μM (Padayatty and Levine 2016).

Many symptoms of scurvy seem to match with recognized roles of ascorbate in enzyme reactions, even if no experimental evidence has really established the link. The earliest symptoms of scurvy include fatigue, lassitude, and weakness that could be due to insufficient carnitine biosynthesis, as well as muscle weakness, anemia, and aching joints (EFSA NDA Panel 2013b; Padayatty and Levine 2016). The main symptoms are skin impairments and injuries by deterioration of elastic tissue, which underline the major role of ascorbate in stability of collagen in connective tissues. Scurvy is then associated with impaired bone growth and ossification in infants; tooth loss, joint pain, bone and connective tissues disorders, and poor wound healing with multiple clinical features in adults such as: bleeding into the skins, subcutaneous tissues, joints, muscles; breakdown of old wounds; bruising; gingival inflammation and bleeding; erythema, purpurea: arthralgia and joint effusions (Fig. 28). People infected by scurvy can also develop depression, mood changes, and

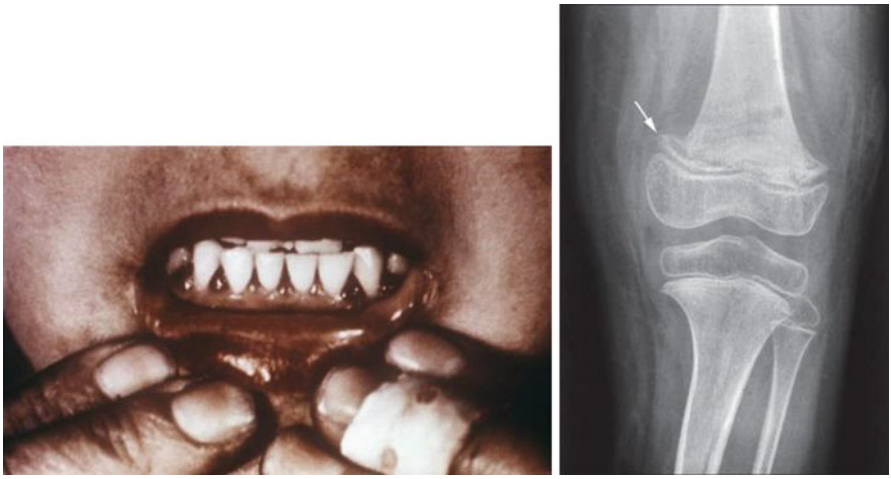


Fig. 28 Scorbutic gums in a patient and scurvy line indicated by an arrow on an X-Ray radiograph of the knee joint (wikipedia)

hypochondriasis, probably due to the impact of ascorbate in dopamine hydroxylation. A dyspnea can be also observed in some cases. The diagnosis is a clinical examination, radiographs with X-rays, and improvement after treatment (Fig. 28). A useful mnemonic for memorize symptoms of scurvy is 4H: hemorrhagic signs, hyperkeratosis, hematologic abnormalities, and hypochondriasis. Multiple vitamin deficiencies, as well as some other diseases such as osteomyelitis, can be confusing. The issue of scurvy is fatal without therapeutic care. The children are treated with vitamin C 100–300 mg daily and adults 500–1000 mg daily for at least 1 month or until symptoms disappear (Agarwal et al. 2015; EFSA NDA Panel 2013b; Padayatty and Levine 2016).

European Food Safety Authority established an Average Requirement (AR) of 90 mg/day for men and 80 mg/day for women, as well as a Population Reference Intake (PRI) of 110 mg/day for men and 95 mg/day for women. The PRI for other populations are specified in the Table 11 (EFSA NDA Panel 2013b). These references are determined on the basis of a plasma concentration around 50 μmol/L of ascorbate, indicative of an adequate status.

The Recommended Dietary Allowance (RDA) was estimated to be 90 mg/day for men and 75 mg/day for women in the USA and Canada. These values were set to maintain near-maximal neutrophil concentration with minimal urinary excretion of ascorbate. The requirement for smokers is increased by 35 mg/day due to the increasing of oxidative stress and metabolic turnover of vitamin C with smoking. The median dietary intakes of vitamin C for adults are estimated to 102 mg/day in the United States and 72 mg/day in Canada (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000).

A significant number of in vitro studies have underlined the physiological role of vitamin C on cellular functions and enzymatic reactions. However, these effects have

Table 11 Dietary reference values of vitamin C from EFSA, expressed in mg/day (EFSA NDA Panel 2013b)

Population	Population references intakes (mg/day)	
	Male	Female
Infants		
0–6 months	–	
7–11 months	20	20
Children		
1–3 years	20	20
4–6 years	30	30
7–10 years	45	45
11–14 years	70	70
Adolescents		
15–17 years	100	90
Adults*		
18–39 years	110	95
Pregnancy		+10
Breastfeeding		+60

not been clearly demonstrated in vivo and remain unclear in clinical conditions, physiologic or pathologic, mainly due to the choice of the employed dose and the sigmoidal dose-concentration relationship of vitamin C (Padayatty and Levine 2016). As we discussed previously in the part "bioavailability," plasma concentrations in humans are firmly controlled when ascorbate is taken orally because of homeostatic regulation. The steady-state plasma concentrations do not exceed 220 μmol/L even if the maximum tolerated oral dose is taken (18 g daily), which may limit the differences between control and treatment groups in clinical trials in terms of plasma and tissue vitamin C concentrations (Graumlich et al. 1997; Levine et al. 1996; Padayatty and Levine 2016).

A significant number of clinical trials exist, however, but with often small effects. The effect of vitamin C has been studied in particular for its role in the prevention, duration, and severity of the **common cold**. A Cochrane meta-analysis concluded that vitamin C was not effective in the prevention of the common cold when taken on a regular basis, at least 200 mg/day, and even at higher doses 1000 mg/day. However, taking vitamin C on a regular basis could reduce, according to the studies, the severity and the average duration, by 8% in adults and 14% in children, of the common cold. A vitamin C supplementation would have more impact on the reduction of incidence of colds among great athletes exposed to brief periods of severe physical exercise such as marathon runners (Hemilä and Chalker 2013). A more recent meta-analysis conducted on children underlined that vitamin C intake has no preventive effects but reduces the duration of upper respiratory tract infections (URTI) (Vorilhon et al. 2019). Due to the low cost and safety of vitamin C, its supplementation could be justified to reduce the duration and severity of common colds in children and adults, as well as upper respiratory tract infections especially in children under 6 years of age and/or submitted to high frequency of URTI (Hemilä and Chalker 2013; Vorilhon et al. 2019). Moreover, EFSA estimated that the dietary

intake of vitamin C contribute to the normal function of the immune system in adults and in children under 3 years of age (EFSA NDA Panel 2009d, 2015b).

Other clinical trials focus in particular on the interest of vitamin C in the prevention and treatment of **cancer**. Some studies deal with the potential interest of a diet rich in vitamin C, through a normal food intake or oral supplementation, on the reduction of the risk of developing cancer. The conclusions of the different meta-analysis are not systematically converging and are rather in the demonstration of the absence of a relationship between dietary vitamin C intake and risk of cancer (Fang et al. 2015; Fulan et al. 2011; Harris et al. 2014; Hua et al. 2016; Long et al. 2019). However, an increasing fruit and vegetable intake, source of vitamin C, not antioxidant supplement use, could globally reduce the risk of chronic diseases such as total cancer and prevent premature deaths. Optimal intakes for chronic diseases prevention could be about 800 g/day for fruits and vegetables, 225 g/day for whole grains, and 15–20 g/day for nuts (Aune 2019; Aune et al. 2018). Other clinical trials relate to the interest of high doses of vitamin C intravenously in the treatment of cancer. Indeed, as already discussed in the part "metabolism," intravenous administration bypasses the homeostatic regulation and plasma concentrations can reach much higher values than by oral route, until 15 mmol/L. At these extracellular concentrations, vitamin C could be toxic for cancer cells, by a prooxidant process via the production of H_2O_2. But, other researchers postulate that vitamin C could act as an antioxidant and reduce then the secondary effects of radiotherapy and chemotherapy (Du et al. 2012; Fritz et al. 2014; Jacobs et al. 2015; Parrow et al. 2013; Wilson et al. 2014). In the current state of the knowledge, no sufficient evidence allows to suggest that ascorbate supplementation by the IV route can be recommended outside of a clinical trial (Jacobs et al. 2015; Wilson et al. 2014). However, recent studies showed a role of vitamin C in regulation of the cancer epigenome. Ascorbate also contributes to enzymatic reactions of histones demethylation and DNA demethylation (Camarena and Wang 2016; Du et al. 2012; Gillberg et al. 2018; Ngo et al. 2019). Prominent preclinical studies supported by well-elaborated clinical trials could bring valuable data to confirm the use of vitamin C by the IV route in the supportive care of cancer patients (Du et al. 2012; Ngo et al. 2019; Vissers and Das 2018).

Vitamin C could also prevent **postoperative atrial fibrillation in cardiac surgery** (Hemilä and Chalker 2019; Hemilä and Suonsyrjä 2017; Putzu et al. 2019; Shi et al. 2018). On the other hand, the results of clinical trials are not conclusive, and sometimes controversial, in the current state of knowledge, concerning the relationship between vitamin C intake and cardiovascular diseases (Al-Khudairy et al. 2017; Ashor et al. 2014, 2019) and between vitamin C intake and cognitive function (Crichton et al. 2013; Li et al. 2012; Travica et al. 2017).

26.4.5 Applications in Food

Vitamin C is mainly provided by plant species, in particular edible fruits and vegetables. Table 12 sums up major plant food sources of vitamin C, mainly according to the French Agency of Food Security (ANSES 2017), selected on the

Table 12 Vitamin C content of plant products, which are rich in vitamin C (ANSES 2017). Only vitamin C content superior to 24 mg/100 g and foods with confidence levels A–C are reported. In some cases, vitamin C content provides from other sources than ANSES 2017. The sources are then specified in footnotes

Category	Food	Total vitamin C (mg/100 g) (min–max values when available)
Algae	Ao-nori (*Enteromorpha* sp.), dried	35.4 (9.24–53.8)
	Dulse (*Palmaria palmata*), dried	83.6 (15.9–187)
	Nori (*Porphyra* sp.), dried	57.3 (2.18–193)
	Rockweed (*Ascophyllum nodosum*), dried	94.8 (0.21–239)
	Sea lettuce (*Ulva* sp.), dried	54.6 (0.28–267)
	Sea thong (*Himanthalia elongata*), dried	62.8 (27.5–133)
	Wakame (*Undaria pinnatifida*), dried	28 (2.84–137)
Fruits	Acerola (*Malpighia emarginata*), raw	**1677**[a]
	Baobab fruit pulp	**280–300**[b]
	Blackberry, raw	36.4
	Blackcurrant, raw	181 (113–255)
	Breadfruit, raw	29
	Camu-camu (*Myrciaria dubia*), raw	**2800 (2400–3000)**[c]
	Cauliflower, frozen, cooked	31.3
	Currant, raw	45 (25–70)
	Ditax fruit pulp (*Detarium senegalense*), raw	**1200–2200**[d]
	Elderberry, berries, raw	32.5 (29–36)
	Goji berries (*Lycium barbarum*, *Lycium chinense*), raw	55.29 (33.15–113.8)[e]
	Gooseberry, raw	29 (18–56)
	Grapefruit, pulp, raw	33.3
	Guava pulp, raw / nectar	**228** (173–283) / 36
	Indian gooseberry (*Phyllanthus emblica*), raw	445[f]
	Kakadu plum (*Terminalia fernandinia*), raw	**3000 (1000–5300)**[g]
	Kiwi, pulp and seeds, raw	92.7 (77.4–118)
	Kumquat seedless, raw	45 (41–49)
	Lemon, zest, raw / pulp, raw / pure juice / homemade juice	**129** / 51 (49–53) / 24.8 / 42.4 (32.1–46)
	Lime, pulp, raw / homemade juice	29.1 / 30 (27.3–33.8)
	Litchi, pulp, raw	71.5
	Mandarin, pulp, raw	26.7 (22.1–34)
	Mango, pulp, raw	32.1 (13.2–92.8)
	Orange, pulp raw / homemade juice / pure juice	57 (53.2–60.8) / 52.6 (45.6–61.7) / 37 (19.1–69)
	Papaya, pulp, raw	61.4 (24.6–93.1)
	Passion fruit, pulp and seeds	30
	Pineapple, pulp, raw	36.4 (7.9–70.7)
	Pink grapefruit, pulp, raw	31.2 (22.6–36.3)

(continued)

Table 12 (continued)

Category	Food	Total vitamin C (mg/100 g) (min–max values when available)
	Pomelo, pulp, raw / homemade juice	61 / 38
	Raspberry, raw / frozen, raw	26.2 (11.2–37) / 24.5 (14–38)
	Red fruits, dried (raspberries, strawberries, currants, blackcurrants)	77.5 (67.9–87)
	Rose hips, wild	**426**[a]
	Sea buckthorn (*Hippophae rhamnoides* subsp. *rhamnoides*), raw	**400**[h]
	Star fruit, pulp, raw	34.4 (24.3–52)
	Strawberry, raw	67.4 (52.5–76)
Grains	Alfafa seeds	26
Nuts and oil seeds	Chestnut, raw / roasted / cooked in water	41.6 (40.2–43) / 26 / 26.7
Spices and herbs	Cayenne pepper	76.4
	Chilli pepper, raw	155 (44.8–245)
Starchy vegetables	Chervil, fresh	37 (10–89)
	Chive, fresh	39.7 (18.8–146)
	Coriander, fresh	27 (10.5–52.5)
	Dill, fresh	70 (8–140)
	"Herbes de Provence," dried	54
	Laurel, leaf	46.5
	Marjoram, dried	51.4
	Onions, dried	75
	Parsley, dried / fresh	137 (125–149) / 177 (89–358)
	Rosemary, dried	61.2
	Sage, dried	32.4
	Saffron	80.8
	Sorrel, raw	48
	Savory, dried	50
	Thyme, dried / fresh	50 /160
Vegetables various	Brocoli, raw / frozen raw / cooked / frozen cooked / mash	106 (85.2–121) / 62.3 (56.3–68.3) / 23.9 / 40.1 / 90
	Brussel sprouts, raw / cooked / frozen, cooked	103 (85–158) / 56.4 / 45.7
	Cabbage, green, raw / cooked	69 (22.9–105) / 27.3 (17–40.5)
	Cabbage, red, raw / cooked in water	58.5 (48–70) / 34.4
	Cabbage, white, raw	45.8
	Chinese cabbage, raw	40.3 (26–45)
	Dandelion, raw	37.5 (35–40)
	Harissa (condiment sauce)	31.9
	Horseradish, raw	152 (102–260)
	Kale, raw / cooked	145 (104–262) / 41
	Kohlrabi, raw / cooked in water	55.1 (48.1–62) / 154

(continued)

Table 12 (continued)

Category	Food	Total vitamin C (mg/100 g) (min–max values when available)
	Leek, frozen, raw	26
	Melon, pulp, raw	28.8 (13–40.7)
	Olives green, stuffed	38.6
	Pea, raw	41.5 (32–59)
	Potato, dehydrated flakes, nature	59 (7.1–83.6)
	Pepper green, raw	92.2 (64.8–134) / 74.4
	Pepper red, raw / cooked	159 (1–210) / 81
	Pepper yellow, raw	184
	Red radish, raw	27.5 (3.8–50)
	Romanesco cabbage	70.6 (53–118)
	Rutabaga, raw	33 (25–56)
	Snow pea, raw	60
	Spinach, raw / deep frozen (raw) / plantlets (raw) / cooked	41.1 (18–86) / 21.4 (0.7–51) / 11.6 / 2.1
	Tomato, dried / dried in oil	39.2 / 102
	Vegetables for couscous, frozen raw	27.4 (24–30.8)
	Vegetables for soups, frozen, raw	25
	Watercress	51.5 (40–80)

[a](USDA 2020)
[b](Kamatou et al. 2011)
[c](Justi et al. 2000)
[d](Diop Ndiaye et al. 2011)
[e](Yossa Nzeuwa et al. 2019)
[f](Tarwadi and Agte 2007)
[g](Brand and Cherikoff 1985)
[h](Gutzeit et al. 2008)

ground of established regulatory thresholds for any food to be considered a source of vitamin C (>12 mg/100 g) or rich in vitamin C (>24 mg/100 g). The most common edible fruits in Europe with higher content of vitamin C are blackcurrant, guava, kiwi, litchi, strawberry, and citrus (orange, lemon, pomelo). Some wild fruits, such are Sea buckthorn and rose hips, are more rich in vitamin C with about 400 mg of vitamin C per 100 g. However, less common tropical fruits have much higher contents, including kakadu plum fruits (3000 mg of vitamin C per 100 g), Camu-Camu fruits (2800 mg of vitamin C per 100 g), and Ditax fruits (with a mean of 1700 mg of vitamin C per 100g). Some of these species are found in supplement foods as source of vitamin C. The most common edible vegetables are peppers (Solanaceae) and different cabbages (Brassicaceae) (Chuah et al. 2008; Domínguez-Perles et al. 2014). Another Brassicaceae species (*Cochlearia officinalis* L.) was commonly named *scurvy-grass*, because it was historically used to cure scurvy on board ships (Chauvet 2018). Some herbs such as parsley and some algae contain also significant contents of vitamin C (ANSES 2017).

Prolonged storage and cooking methods may reduce vitamin C content in food (Zhao et al. 2019). Among different home cooking procedures (boiling in water, steaming, pressure steaming, microwave heating and stir-frying without using water), the less suitable method seems to be boiling, which leads to high loss of vitamin C, in particular due to the high solubility of ascorbic acid in water. Microwave heating and stir-frying without using water seems to be more appropriate to preserve ascorbic acid (Bureau et al. 2015; Chuah et al. 2008; Lee et al. 2018). Indeed, thermal treatment can accelerate the conversion of ascorbic acid to dehydroascorbic acid, followed mainly by the hydrolysis of 2,3-diketogulonic acid (Barros et al. 2011; Chuah et al. 2008). Before cooking, it is best not to thaw frozen vegetables in order to prevent vitamin C to degradation (Nursal and Yücecan 2000).

26.4.6 Safety: Toxicity and Side Effects

The Panel on Dietary Antioxidants and Related Compounds in the USA and Canada determined a Tolerable Upper Intake Level (UL) for adults at 2 g/day. This UL is based on some adverse effects such as osmotic diarrhea and gastrointestinal disturbances (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000). On the other hand, EFSA did not determine a UL. Vitamin C seems to be a low acute toxicity according to available studies conducted in animals. The agency also notes that the most defined side effects at high doses (3–4 g/day) are reversible gastrointestinal intolerance and diarrhea, due to the degradation of unabsorbed ascorbate by the intestinal microflora. Some renal effects can be observed due to the urinary excretion of oxalic acid, in particular in individuals with impaired renal function. As discussed above, vitamin C also could exert a prooxidant effect in the presence of catalytic metals leading to the formation of free radical through the Fenton reaction. However, the link between these effects and an excess of vitamin C intake is not clearly established (Du et al. 2012; EFSA NDA Panel 2013b).

26.4.7 Marketed Products

According to reference website for healthcare professionals in France, ascorbic acid can be marketed orally in some pharmaceutical specialties alone at a dose of 1 g per tablet or in association with sodium ascorbate sodium to treat asthenia or vitamin C deficiency. But, ascorbic acid can be also associated, at doses varying between 100 mg and 500 mg per tablet, with other active ingredients to manage different diseases such as for example quinine to take care muscle cramps, with arginine aspartate to manage asthenia, with paracetamol and antihistamines to manage fevers and nasopharyngitis, and with citroflavonoids or rutoside/α-tocopherol to treat hemorrhoids and venolymphatic insufficiency. Ascorbic acid can be also proposed in solution for intravenous injection (1 g/5 ml) to treat vitamin C deficiency (Vidal 2013).

Ascorbic acid can also be found in food supplements, alone, in combination with other vitamins and minerals or in combination with antioxidant plant extracts. The proposed doses are in general 500 or 1000 mg per tablet. Vitamin C can be synthetic or with natural origin. Different fruits with high content of vitamin C are marketed in food supplements, such as acerola, rose hips, camu-camu, and goji fruits.

Unlike animal studies, all bioavailability studies conducted in humans have demonstrated no differences between synthetic and natural vitamin C, whatever the methodology used (subject population, study design, intervention used). The few differences observed in pharmacokinetic studies in humans seem to have a minimal physiological impact (Carr and Vissers 2013).

Oral liposomal vitamin C formulations have been developed to improve its stability and enhance its bioavailability (Caritá et al. 2020; Davis et al. 2016; Łukawski et al. 2019).

26.4.8 Patents

A considerable number of patents have been filed on vitamin C since its isolation and identification in the 1930s. The patents relate in particular to optimization of its synthesis, development of methods of extraction from natural sources, microbial production, biotechnological production, development of analytical methods for detection and identification of vitamin C in different matrix, development of galenic formulations to improve the stability, and the bioavailability of vitamin C for pharmaceutical, agro-food, or cosmetic applications.

26.4.9 Perspectives

The ongoing interest for ascorbic acid-related disorders in major health concerns such as cancer show that various aspects in its biological roles are still to be deepened and underline the potential of vitamin C intake intravenously. Moreover, the sigmoidal dose-concentration relationship of vitamin C when taken orally should not to be overlooked in clinical trials. More fundamental research is also needed to provide evolutionary features to explain the lack of vitamin C synthesis in some mammals, in particular humans.

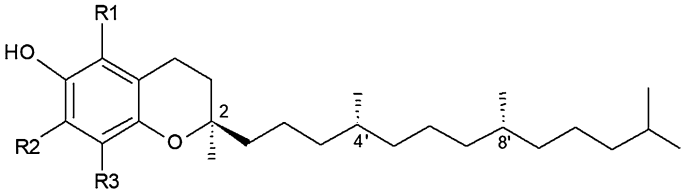
26.5 Vitamin E: α-Tocopherol

26.5.1 Bioactive Constituents

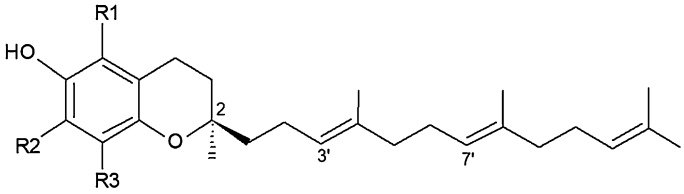
Vitamin E is a liposoluble vitamin which can be considered as a mixture of eight naturally occurring forms, including four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ), called tocochromanols. Their structure, relatively simple, is composed of a polar hydroxylated ring system (chromanol ring) and a long apolar isoprenoid side

chain. The hydroxyl group carried by the chromanol ring can donate a hydrogen atom to reduce free radicals, whereas the hydrophobic side chain permits the penetration of the vitamin into biological membranes. The different forms (α, β, γ, δ) are determined by the number and position of methyl groups on the chromanol ring. Tocopherols differ from tocotrienols only by the nature of the hydrophobic chain. The saturated phytyl side chain of tocopherols is replaced by a geranylgeranyl side chain, an unsaturated chain with three double bonds. Tocopherols have three chiral centers in positions 2, 4′ and 8′, leading to the formation of eight possible stereoisomers, but only the *RRR* stereochemistry of tocopherols occurs naturally in plants (Fig. 29). The naturally occurring *RRR*-α-tocopherol (formerly and incorrectly named *d*-α-tocopherol) is the unique physiologically active form because is maintained in human plasma by means of its preferential interaction with liver α-tocopherol transfer protein (α-TTP). Thus, some agencies, such as EFSA, consider vitamin E as being α-tocopherol only, with a molecular mass of 430.71 Da. Synthetic vitamin E, all racemic- (*all rac*)-α-tocopherol (formerly and incorrectly called *dl*-α-tocopherol), contains the eight stereoisomers of α-tocopherol in equal amounts. Only 2*R*-stereoisomeric forms (*RRR*-, *RSR*-, *RRS*, and *RSS*-α-tocopherol) are maintained in human plasma; the 2*S*-stereoisomeric forms (*SRR*-, *SSR*-, *SRS*, and *SSS*-α-tocopherol) have low affinity to α-TTP and are rapidly metabolized in liver (Azzi 2019; EFSA NDA Panel 2015c; Fritsche et al. 2017; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000). Tocotrienols in plants are found with one asymmetric center in the R configuration (2R) and three double bonds, two of them, in positions 3′ and 7′, are in the "E" configuration. Historically, vitamin E was discovered in 1922, isolated in 1935, identified and first synthesized in 1938. Because the first activity for this vitamin was recognized as essential for fertility and fetal development in rats, the name "tocopherol" was given and comes from the Greek *tókos* meaning "birth" and *phérein* meaning "to bear or carry" (Evans and Bishop 1922; Fernholz 1938; Karrer et al. 1938). However, the essentiality of this function has never been confirmed in humans, except for one case report reporting the successful birth in woman with recurrent spontaneous abortions after administration of tocopherol nicotinate 300 mg/day (EFSA NDA Panel 2015c; Harada et al. 2005).

By its hydroxyl group on the chromanol ring, vitamin E has the ability to capture and stabilize by resonance the electron of free radicals in membranes and in plasma lipoproteins. It is a chain-breaking antioxidant and a peroxyl radical scavenger which mainly preserves the integrity and thus the bioactivity of long-chain polyunsaturated fatty acids in membranes of cells. It protects fatty acids from lipid peroxidation (Traber and Atkinson 2007). Thus, α-tocopherol scavenges lipid peroxyl radical ($LOO^{\bullet}$) before it attacks lipids. α-tocopherol is converted to α-tocopheroxyl radical ($E^{\bullet}$), which is resonance-stabilized, and a lipid peroxide (LOOH) is formed. The resulting vitamin E radical ($E^{\bullet}$) may attack lipids and react with another radical such as $LOO^{\bullet}$ or $E^{\bullet}$ to provide an adduct or a dimer. Vitamin E radical ($E^{\bullet}$) may also react with ascorbate or other hydrogen donors to form α-tocopherol to its reduced state, illustrating the vitamin E recycling (Fig. 30). Due to the fact that vitamin E to its reduced state is continuously restored, oxidized tocopherols emphasized in vitro are usually not found in vivo (Buettner 1993; Niki 2014; Traber and Stevens 2011).



α-tocopherol : R1 = CH_3; R2 = CH_3; R3 = CH_3
β-tocopherol : R1 = CH_3; R2 = H; R3 = CH_3
γ-tocopherol : R1 = H; R2 = CH_3; R3 = CH_3
δ-tocopherol : R1 = H; R2 = H; R3 = CH_3



α-tocotrienol : R1 = CH_3; R2 = CH_3; R3 = CH_3
β-tocotrienol : R1 = CH_3; R2 = H; R3 = CH_3
γ-tocotrienol : R1 = H; R2 = CH_3; R3 = CH_3
δ-tocotrienol : R1 = H; R2 = H; R3 = CH_3

Fig. 29 Structures of tocopherols and tocotrienols. All tocopherols are represented in the *RRR*-forms. (Adapted from (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000)

Vitamin E is an essential dietary constituent, because it cannot be produced in humans and in animals. As plants biosynthesize tocochromanols, the major sources in human nutrition are vegetable oils. Cereals, nuts, and green leafy vegetables can also provide vitamin E in diet. Tocochromanols occur exclusively in photosynthetic organisms including plant species, green algae, and some cyanobacteria (Mène-Saffrané and DellaPenna 2010). Tocochromanols also include plastochromanol-8 (PC8) which is not covered in this chapter, because it is not directly included in vitamin E (Kruk et al. 2014). The most abundant form of tocopherol isolated from photosynthetic tissues of many plant species, in particular in eudicots, as well as in cyanobacteria, is α-tocopherol in the *RRR* stereochemistry. However, α-tocopherol can be accumulated in seeds of some eudicots species, such as *Arabidopsis thaliana*. Unlike α-tocopherol, tocotrienols are less widespread in the plant kingdom. They are most abundant in monocot species, such as Poaceae and Arecaceae species, and are rare in eudicots. They are relatively rare in photosynthetic tissues and are more abundant in seeds and in latex. The tocochromanol biosynthetic pathway has been particularly studied in *Arabidopsis* and *Synechocystis* species. The major subcellular localization of tocochromanols in plant cells is plastids, such as leaf chloroplasts and seed plastids, even if other localizations have been highlighted including seed lipid bodies and mesophyll cells. As in animals, the major role of vitamin E is to maintain

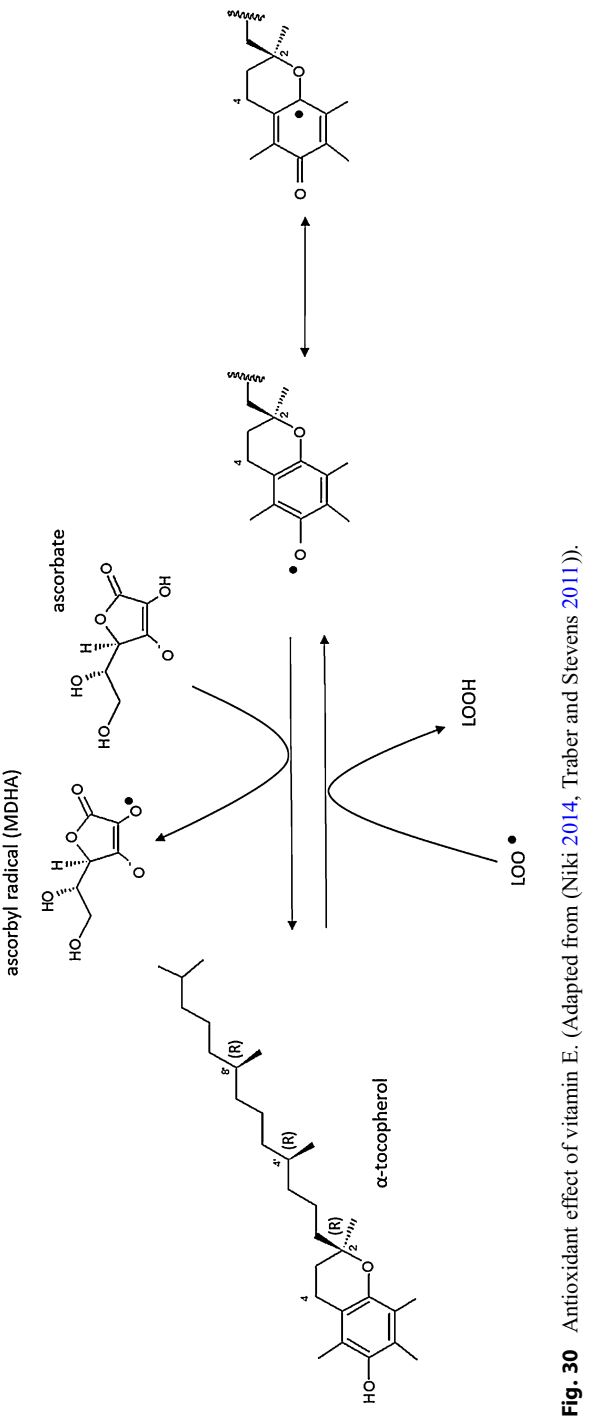


Fig. 30 Antioxidant effect of vitamin E. (Adapted from (Niki 2014, Traber and Stevens 2011)).

membrane integrity and stability by its protective effect against propagation of lipid peroxidation. Vitamin E can also contribute to the modulation of ROS accumulation and then play a role in photoprotection when leaves are subjected to photo-oxidative stress. This effect associated with modulation of lipid peroxidation can be linked to the role, recently underlined, of vitamin E in stress sensing and intracellular signaling (Muñoz and Munné-Bosch 2019).

Tocochromanol biosynthesis takes place in the plastids of plant cells, except for the first step that can be initiated in the cytoplasm (Fig. 31). This first step, common to all tocochromanols, derives from the shikimate pathway and consists of converting *p*-4-hydroxyphenylpyruvate (HPP) to homogentisate (HGA) via the *p*-4-hydroxyphenylpyruvatedioxygenase (HPPD) (Dörmann 2007; Fritsche et al.

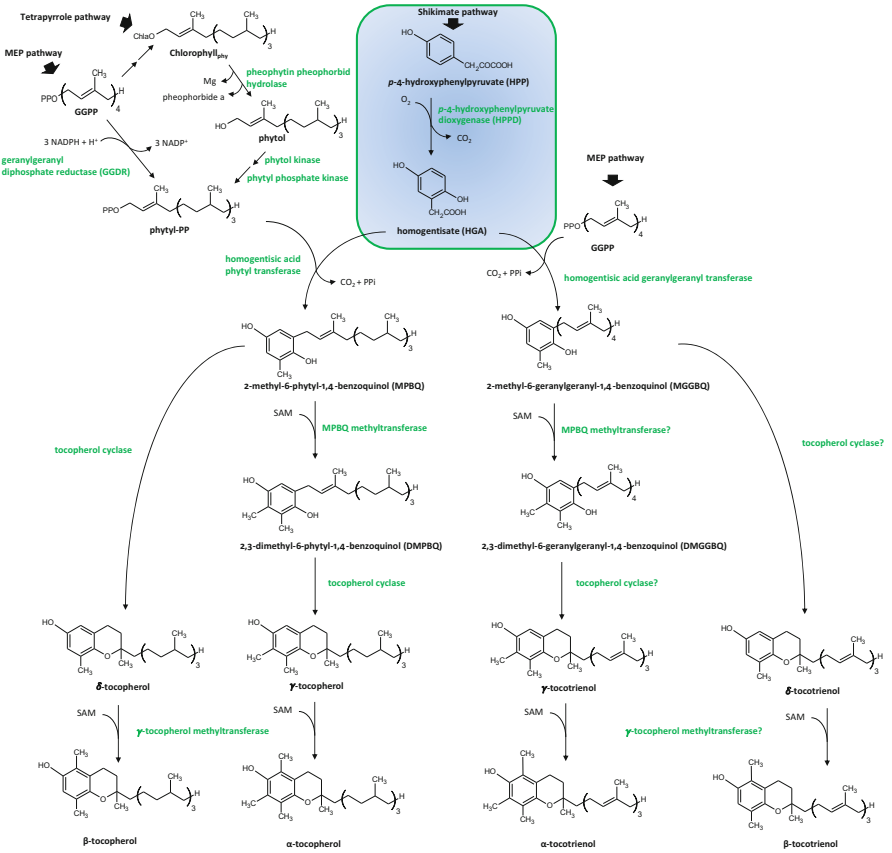


Fig. 31 Tocopherol and tocotrienol biosynthesis pathways. Adapted from (Fritsche et al. 2017; Mène-Saffrané and DellaPenna 2010; Muñoz and Munné-Bosch 2019). The first step framed in green is common of the two classes of tocochromanols. Enzymes implicated in tocopherol biosynthesis pathway were identified in *Arabidopsis thaliana*. The enzymes involved in the last steps of tocopherol synthesis, in particular methyltransferases and tocochromanol cyclases, could be the same as that involved in tocotrienol synthesis.

2017). The tocochromanol biosynthesis differs at the second step where a polyprenyl side chain is attached to HGA. This polyprenyl side chain derives mainly from isopentenyl diphosphate (IPP) from the methylerytrithol phosphate pathway (MEP) from 1-deoxy-D-xylulose 5-phosphate. Geranygeranyl diphosphate (GGPP) will be at the origin of the side chain of tocotrienols. On the other hand, phytyl-PP involved in tocopherol biosynthesis may have two origins. It can be provided by the reduction of GGPP by a geranylgeranyl diphosphate reductase or mostly by the recycling of free phytol derived from chlorophyll degradation. Phytol is released during chlorophyll breakdown via the intermediate chlorophyll$_{phy}$, chlorophyll linked to a phytyl moiety. This alternative metabolic way, known as the phytol recycling pathway, occurs in plants both in responses to environmental stresses and during developmental processes such as leaf or flowers senescence, seed maturation, or ripening of fruits (Fritsche et al. 2017; Mène-Saffrané and DellaPenna 2010; Muñoz and Munné-Bosch 2019; vom Dorp et al. 2015). In tocopherol biosynthesis, HGA is condensed with phytyl-PP by means of homogentisic acid phytyl transferase to form 2-methyl-6-phytyl-1,4-benzoquinol (MPBQ), which will be the common precursor to all tocopherols. MPBG can then be directly cyclized by a tocopherol cyclase to form α-tocopherol. It can also be methylated in position 3 by the MPBQ methyltransferase to form 2,3-dimethyl-6-phytyl-1,4-benzoquinol (DMPBQ) which will then be cyclized by a tocopherol cyclase to form γ-tocopherol. Finally, δ- and γ-tocopherols are methylated at the position C-5 by the γ-tocopherol methyltransferase to form β- and α-tocopherols, respectively.

In tocotrienol biosynthesis conducted mainly in seeds of monocots, HGA is condensed with GGPP by means of homogentisic acid geranylgeranyl transferase to form 2-methyl-6-geranylgeranyl-1,4-benzoquinol (MGGBQ), which will be the common precursor to all tocotrienols (Yang et al. 2011). The enzymes responsible for the methylation of MGGBQ into DMGGBQ, the cyclization of MGGBQ, and DMGGBQ into δ- and γ-tocotrienols, respectively, as well as their following methylation into β- and α-tocotrienols have not been yet reported from tocotrienols producing species. The enzymes involved in the last steps of tocopherols synthesis, in particular methyltransferases and tocochromanol cyclases, could be the same as that involved in tocotrienols synthesis (Fritsche et al. 2017; Mène-Saffrané and DellaPenna 2010; Muñoz and Munné-Bosch 2019).

26.5.2 Bioavailability and Metabolism

26.5.2.1 Bioavailability

Absorption

After ingestion, vitamin E is released from its food matrix, dissolved in the lipid phase of the bolus at gastric and duodenal levels, and then incorporated into lipid droplets. In the duodenum, vitamin E is combined by bile salts with other lipid digestion products mostly into mixed micelles, which can be diffused through the

unstirred water layer of the glycocalix area to reach the apical membrane of the enterocytes. Several factors can impact the vitamin E transfer to mixed micelles such as food matrix, as well as quantity and quality of fat and fat-soluble micronutrients provided by the meal (Borel et al. 2013b; EFSA NDA Panel 2015c; Reboul 2017, 2019b). Along with free tocopherols, tocopherol esters can be also provided by food supplementation and must be hydrolyzed in the lumen of the small intestine before absorption. This hydrolysis could be led by some pancreatic enzymes whose activity requires the presence of bile salts such as cholesteryl ester hydrolase, also known as bile salt-dependent lipase. Other pancreatic enzymes, including pancreatic lipase and pancreatic lipase-related protein 2, are not able to contribute to this hydrolysis. The absorption of the free and esters forms are similar in healthy subjects even in absence of digestive enzymes and bile salts, suggesting that other enzymes from enterocytes, such as endoplasmic reticulum esterases, could contribute to the hydrolysis of tocopherol acetates in an efficient manner (Desmarchelier et al. 2013; Lombardo and Guy 1980; Mathias et al. 1981; Nagy et al. 2013; Reboul 2017). Until fairly recently, vitamin E was thought to be absorbed only by passive diffusion, after dissociation of mixed micelles, through enterocyte apical membrane by transporters non-specific to tocopherols. However, after vitamin E intake, postprandial responses are very variable in humans. Some recent advances show that the absorption of α- and γ-tocopherol is in reality more complex and involves at least partially different receptors and transporters, in particular Scavenger Receptor Class B Type 1 (SR-B1), CD36 molecule, and NPC1-like intracellular cholesterol Transporter 1 (NPC1L1) (Reboul 2017, 2019b). No studies determine precisely the quantification of α-tocopherol in humans. Former studies using radioactive or deuterium-labeled α-tocopherol give variable results with a large range of reported mean α-tocopherol absorption, from about 10% to 80% for different fat intakes. In 2015, on the basis of some studies, the EFSA Panel estimated an average α-tocopherol absorption from a normal diet about 75% (Borel et al. 2013b, 2015; Traber and Stevens 2011). However, new insights underline the need to deepen our knowledge on vitamin E absorption in humans.

After intestinal absorption, tocopherols are incorporated in intestinal epithelial cells into nascent chylomicrons or into high-density lipoproteins (HDL) via the ATP-binding cassette transporter ABCA1. However, only α-tocopherol is maintained in plasma and tissues. α-tocopherol is secreted into the systemic circulation from chylomicrons along the lymphatic pathway. The plasma half-life of α-tocopherol is long, between 48 h and 60 h. By contrast, the plasma half-life of synthetic form is much shorter. The long plasma half-life of *RRR*-α-tocopherol is partly due to its rapid recirculation from the liver to the plasma. Remaining chylomicrons, containing newly absorbed vitamin E, are first captured by the liver. Vitamin E is then secreted from the liver in circulating lipoproteins, by its incorporation thanks to α-tocopherol transfer protein (α-TTP) into nascent very-low density lipoproteins (VLDL) in particular and in high density lipoproteins (HDL) (Galli et al. 2017; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Kayden and Traber 1993; Traber and Stevens 2011). The selective accumulation of α-tocopherol and the facilitated exchange between liver and plasma is thus

explained by preferential affinity of α-TTP, a hepatic cytosolic protein, for α-tocopherol. The relative binding affinities of α-TTP for tocopherols are 100% for natural α-tocopherol, 38% for β-tocopherol, 9% for γ-tocopherol, and 2% for δ-tocopherol (Hosomi et al. 1997). α-TTP also protects α-tocopherol from excessive catabolism mediated by cytochrome P-450, in particular the degradation of α-tocopherol to α-carboxyethyl hydroxychromanol (α-CEHC) mediated by tocopherol-ω-hydroxylase. α-tocopherol bound to α-TTP will be less subject to metabolic degradation by tocopherol-ω-hydroxylase in comparison with other non-α-tocopherol congeners (Galli et al. 2017). Once secreted into the bloodstream, VLDL are converted into intermediate-density lipoproteins (IDL) and low density lipoproteins (LDL) by means of a lipoprotein lipase. The excess of VLDL surface constituents, including α-tocopherol, is moved to high-density lipoproteins (HDL) (Fig. 32) (EFSA NDA Panel 2015c).

Distribution

From lipoproteins, vitamin E is distributed into human extrahepatic tissues. α-tocopherol is the vitamin E form found at highest concentrations, followed by γ-tocopherol. Tocotrienols are habitually not detected in tissues (Galli et al. 2017). α-tocopherol is particularly detected in adipose tissues with more than 90% of the total body *RRR*-α-tocopherol pool. The α-tocopherol delivery to peripheral tissues can be explicated by at least two mechanisms: the release of α-tocopherol during the hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase and a receptor-uptake of LDL- and HDL-bound α-tocopherol (Fig. 32) (EFSA NDA Panel 2015c; Galli et al. 2017; Rigotti 2007).

26.5.2.2 Metabolism

The liver plays an essential role in the recirculation and metabolism of vitamin E, in particular α-tocopherol. In phase I, tocopherols and tocotrienols are metabolized by ω-hydroxylation in endoplasmic reticulum, followed by ω-oxidation and β-oxidation in peroxisome and mitochondrion to provide long-chain and intermediate-chain metabolites before the final formation of the catabolic end-products which are short-chain metabolites known as carboxy-ethyl-hydroxy-chromanols (CEHC). α-tocopherol will be transformed for example in α-CEHC (Fig. 33). In phase II, these metabolites can be then conjugated, sulfated, or glucuronided. All metabolites can be found in feces, whereas only short-chain metabolites will be found in urines (EFSA NDA Panel 2015c; Galli et al. 2017; Mustacich et al. 2010; Schmölz 2016). As previously raised, the degradation of α-tocopherol is less subject to the catabolic activity of tocopherol-ω-hydroxylase than other tocopherols and tocotrienols thanks to α-TTP. α-tocopherol bound to α-TTP is not catabolized by tocopherol-ω-hydroxylase, which has thus a stronger activity towards other tocochromanols. α-tocopherol will be thus preferentially distributed in tissues, while the other tocochromanols will be predominantly catalyzed in the liver (EFSA NDA Panel 2015c).

Sulfate and glucuronide conjugates will be found in urines and feces, but feces remain the main route of elimination of vitamin E. Some recent in vitro and in vivo studies underlined the regulatory potential of vitamin E metabolites, in particular

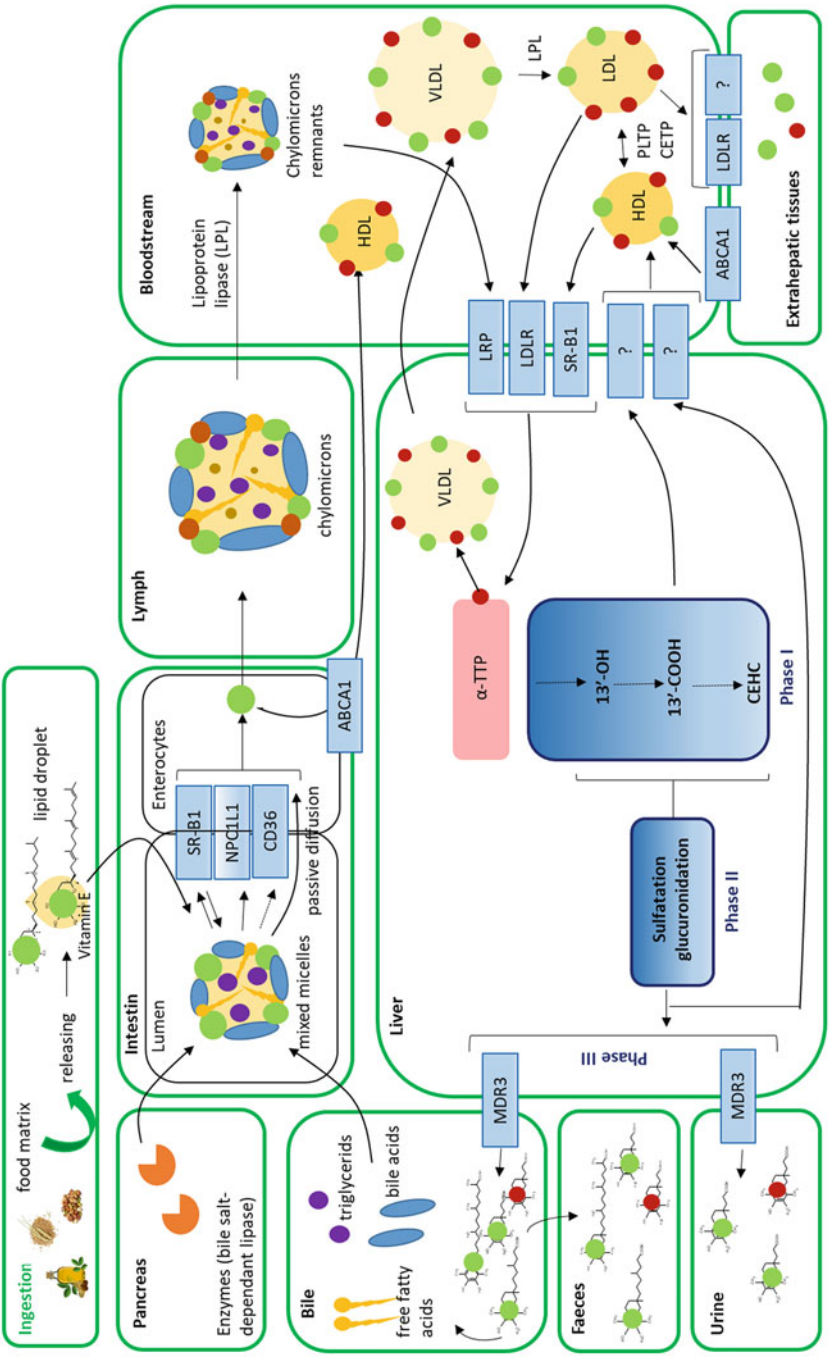


Fig. 32 (continued)

long-chain metabolites from γ-tocopherol and tocotrienols, in inflammatory processes (Birringer and Lorkowski 2019; Jiang 2014; Schmölz 2016). α-tocopherol is secreted into breast milk at about 4 mg/day during the first 6 months of exclusive breastfeeding (EFSA NDA Panel 2015c).

26.5.3 Bioactivities

26.5.3.1 General Role

Vitamin E as a Chain-Breaking Antioxidant

Vitamin E mainly operates as a chain-breaking antioxidant and thus prevents the propagation of free-radical reactions. It is especially implicated in lipid peroxidation, which consists of an oxidative degradation of lipids. Vitamin E is a peroxyl radical scavenger and particularly protects polyunsaturated fatty acids (PUFAq) within membrane phospholipids and plasma lipoproteins. Indeed, lipid peroxyl radicals formed react 1000 times faster with α-tocopherol than PUFAs. The antioxidant mechanism follows the mechanism already described in the first part "Bioactive molecules" (Fig. 30). α-tocopherol radicals and lipid hydroperoxide are thus formed after scavenging of lipid peroxyl radicals by α-tocopherol. α-tocopherol radicals formed may react with another radical to provide steady compounds, attack lipids, or react with an aqueous reducing agent such as ascorbate, selenium, or ubiquinol. This last reaction allows to continuously restore the antioxidant activity of vitamin E. By protecting PUFAs within membranes, α-tocopherol conserves membrane integrity and stability and has a significant role in the stability of erythrocytes and the protection of nervous tissues from oxidative stress. Vitamin E thus prevents

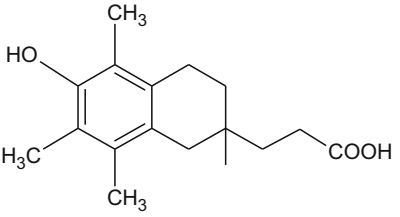


Fig. 33 α-CEHC, a major metabolite of α-tocopherol in the liver

Fig. 32 Absorption, distribution, and metabolism of vitamin E. Adapted from (Galli et al. 2017; Reboul 2019b; Schmölz 2016). ABCA1, ATP-binding cassette transporter; α-TTP, α-tocopherol transfer protein; CETP, cholesteryl ester transfer protein; 13'-COOH, 13'-carboxychromanol; HDL, high density lipoproteins; NPC1L1, Niemann-Pick C1-like intracellular cholesterol Transporter 1; 13'-OH, 13'-hydroxychromanol; PLTP, phospholipid transfer protein; SR-B1: Scavenger Receptor Class B Type 1; LDL, low density lipoproteins; LDLR, LDL receptor; LRP, LDL receptor-related proteins; VLDL, very low density lipoproteins.

hemolytic anemia and neurological symptoms occurring in patients deficient in vitamin E (EFSA NDA Panel 2015c; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Traber and Stevens 2011).

Vitamin E as a Modulator of Signal Transduction and Gene Expression

A number of studies using different cell model systems underlined that vitamin E has also non-antioxidant properties. Indeed, the eight naturally occurring forms of vitamin E affect cell signaling and gene expression. Their potential of modulation is not equivalent, despite an equal capacity to chemically scavenge free radicals. Therefore, the differences observed in signaling effects for each natural vitamin E analogue would not be due only to a general antioxidant action, but could be explained by their differences in bioavailability and mostly by differential binding to enzymes, receptors, transcription factors, and membrane microdomains (Azzi 2018, Zingg 2015, 2019). *RRR*-α-tocopherol has notably the ability to modulate the functional activities of several enzymes directly implicated in cell signal transduction, leading to modifications of cellular behavior such as adhesion, apoptosis, autophagocytosis, differentiation, gene expression, immunity, inflammation, metastasis, migration, proliferation, secretion, senescence, and survival. Vitamin E affects the activity of specific enzymes such as protein kinases and phosphatases, lipid kinases and phosphatases, lipid metabolic enzymes, and enzymes involved in cAMP metabolism (Table 13). The cellular effects described are in general inhibitory and vitamin E may act by different mechanisms including direct binding to these enzymes, modulation of their redox regulation, and modification of their level

Table 13 Modulation of enzymatic activity of specific enzymes involved in signal transduction by vitamin E, according to (Zingg 2015)

Enzyme class	Enzymes modulated by vitamin E
Protein kinases	Protein kinase C (PKC)
	Protein kinase B (PKB/Akt)
	Protein tyrosine kinases (PTKs)
Protein phosphatases	Protein phosphatase 2A (PP2A)
	Pleckstrin homology domain leucine-rich repeat protein phosphatase, isoform 1 (PHLPP1)
	Protein tyrosine phosphatase (PTP)
Lipid kinases	Diacylglycerol kinase (DAGK)
	Phosphatidylinositol-3-kinase alpha (PI3Kα)
	Phosphatidylinositol-3-kinase gamma (PI3Kγ)
Lipid phosphatases	5′- and 3′-Inositol phosphatases (SHIP)
Lipid metabolic enzymes	5-, 12-, 15-lipoxygenases (5-, 12-, 15-LOX)
	Cyclooxygenase-2 (COX-2)
	Phospholipase A2 (PLA2)
Enzymes involved in cAMP metabolism	Adenylyl cyclase
	Phosphodiesterase

expression. *RRR*-α-tocopherol has in particular a central role in the modulation of protein kinase C (PKC) activity through several mechanisms. This negative regulation is the cause of inhibition of cell proliferation of different cell types including brain cells, cancer cell lines, fibroblasts, vascular smooth muscle cells, macrophages, mesangial cells, monocytes, macrophages, and neutrophils. The modulation of PKC activity by *RRR*-α-tocopherol is linked to pathophysiological situations among others inflammation, diabetic vascular complications and platelet aggregation (Azzi 2018; Brigelius-Flohé and Traber 1999; Cachia et al. 1998; Pédeboscq et al. 2012; Schneider 2005; Tasinato et al. 1995; Zingg 2015).

Vitamin E is also able to regulate the expression of genes involved in cellular signaling. The regulatory effect of vitamin E on many transcription factors often goes through an indirect mechanism, such as the modulation of signal transduction enzymes implicated in the regulation of their activity. However, in some cases, vitamin E is able to directly interact with the transcription factor and activate it. It is the case for pregnane X receptor transcription factor and for the estrogen receptor beta. The regulation of gene expression may lead up to the activation of additional signaling cascades as a secondary response (Table 14) (Zingg 2007, 2015).

The regulation of signal transduction by vitamin E can be also influenced by other mechanisms such as direct binding of vitamin E to transport proteins involved in signal transduction, modulation of membrane-protein interaction and protein translocation to the plasma membrane, modulation of plasma membrane properties, modulation of cell surface exposition of membrane receptors, modulation of transport and conversion of lipids to signaling mediators, conversion of vitamin E to active metabolites and lipid mediators, and conversion of cytochrome c into a peroxidase by α-tocopheryl phosphate (Zingg 2015, 2019). The modulation of CD36 scavenger receptor/fatty acids transporter by vitamin E may in particular influence many cellular signaling pathways relevant for physiological and pathophysiological cellular functions (Zingg 2019).

Table 14 Modulation of transcription factors by vitamin E, according to (Zingg 2015).

Transcription factor	Effect of vitamin E
Peroxisome proliferator-activated receptor gamma (PPARγ)	Upregulation of expression and increase of activity by indirect mechanisms
Nuclear factor erythroid-derived 2-like 2 (NRF2)	Upregulation of expression by indirect mechanisms
Nuclear factor kappa B (NFκB)	Inhibition of activation by indirect mechanisms
RAR-related orphan receptor alpha (RORα)	Downregulated with vitamin E deficiency, indirect mechanisms
Hypoxia-inducible factor 1 alpha (Hif1α)	Inhibition of activation by indirect mechanisms
Estrogen receptor beta (ERβ)	Direct binding of tocopherols and increase of activity
Pregnane X receptor (PXR)	Direct binding of tocopherols and increase of activity

Vitamin E as an Anti-inflammatory Agent

α-tocopherol is also known to have anti-inflammatory effects both in vitro and in vivo. At high doses, it decreased the release of some pro-inflammatory cytokines and decreases adhesion of monocytes to endothelium (Singh and Jialal 2004).

Despite these new findings, European Food Safety Authority (EFSA) authorized for the moment a unique health claim for food and food supplements containing at least 1.8 mg of vitamin E per 100 g, based on the cause–and-effect relationship between dietary intake of vitamin E and some biological functions: "vitamin E contributes to the protection of cell constituents from oxidative damage" (EFSA NDA Panel 2010e).

26.5.3.2 Animal Studies

There are of course numerous in vivo studies reporting the biological activities of vitamin E. The first study recognizing the essential role of vitamin E in fertility and fetal development in rats dates from 1922 (Evans and Bishop 1922). Vitamin E, by its antioxidant function, could protect the fetus during the period when it is most vulnerable by oxidative stress (Schneider 2005). However, this role has not been clearly established in humans. The role of vitamin E as a peroxyl radical scavenger and inhibitor lipid peroxidation was clearly highlighted experimentally in rats intoxicated with carbon tetrachloride (Corongiu et al. 1986; Niki 2014).

Some animal models were developed such as α-TTP knock-out mice (α-TTP -/- mice) for which the α-TTP has been made inactive by gene mutations (Azzi 2018). These mice were used to study the link between vitamin E deficiency and diseases in which oxidative stress play a role, such as atherosclerosis or neurodegenerative disorders. In order to consider the impact of vitamin E deficiency on the severity of atherosclerosis, these mice were crossed with apoE −/− mice (Schneider 2005; Terasawa et al. 2000; Ulatowski et al. 2014; Yokota et al. 2001). However, a lack of in vivo evidence of the necessary recycling of vitamin E and a lack of evidence of antioxidant damage in animal models in vitamin E deficiency are enquiring. In these models, no compensatory induction of the endogenous antioxidant system was observed. Moreover, despite a large number of significant clinical trials, few studies have shown a link between vitamin E and beneficial effects against oxidative stress based diseases (Azzi 2018).

26.5.4 Benefits (Human Studies)

Vitamin E deficiency is relatively rare and symptoms were not reported in individuals without any specific disease or with low intake of vitamin E in diet. Symptoms were mostly observed in population with familial isolated α-tocopherol deficiency (primary α-tocopherol deficiency) with mutations of genes coding for α-TTP or in patients unable to absorb correctly the vitamin due to different pathologies (secondary α-tocopherol deficiency) such as abetalipoproteinemia, cholestatic liver diseases, severe malnutrition, malabsorption, and cystic fibrosis. In these cases, serum α-tocopherol concentrations are low, in particular in patients with primary α-

tocopherol deficiency, in whom concentrations can go below 2.3 μmol/L. Clinical manifestations of vitamin E deficiency in humans can be characterized by hemolytic anemia, impairment of immune responses and mainly neurological symptoms such as ataxia, peripheral neuropathy with degeneration of large-caliber axons in sensory neurons, myopathy, and pigmented retinopathy. Adequate intake of vitamin E seems to be important for normal development in infants and young children (EFSA NDA Panel 2015c; Guggenheim et al. 1982; Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000; Nakagawa et al. 1984; Sokol et al. 1985; Traber and Stevens 2011).

Dietary reference values of vitamin E are mainly based on observed intakes. Previously, the Scientific Committee for Food used the generic term, vitamin E, for both tocopherols and tocotrienols. The content of vitamin E had been presented in α-tocopherol equivalents (α-TEs) (Scientific Committee for Food 1993). In the more recent report, based on available evidence and in line with other instances, EFSA Panel considers vitamin E as α-tocopherol only, including the naturally occurring form (*RRR*-α-tocopherol) and the other three synthetic 2R-stereosisomer forms (*RSR*-, *RRS*- and *RSS*-). Adequate intakes (AIs) were determined for children and adults on the basis of observed intakes of α-tocopherol and α-tocopherol equivalents in healthy populations with no apparent α-tocopherol deficiency in the EU. For infants (7–11 months), AIs are provided on the basis of assessed intakes in fully breast-fed infants followed by an extrapolation (Table 15). The Panel estimated that Average Requirements (ARs) and Populations Reference Intakes (PRIs) for vitamin E (as α-tocopherol) cannot be derived for adults, infants, and children (EFSA NDA Panel 2015c)

The Recommended Dietary Allowance (RDA) was estimated to be 15 mg/day (35 μmol/day) of α-tocopherol for both men and women in the USA and Canada.

Table 15 Dietary reference values for α-tocopherol from EFSA, expressed in mg/day (EFSA NDA Panel 2015c)

	Adequate intakes (mg/day)	
Population	Male	Female
Infants		
0–6 months	-	
7–11 months	5	5
Children		
1–3 years	6	6
4–6 years	9	9
7–10 years	9	9
11–14 years	13	11
Adolescents		
15–17 years	13	11
Adults*		
18–39 years	13	11
Pregnancy		11
Breastfeeding		11

Like EFSA Panel, in order to establish the human requirement for vitamin E, the Institute of Medicine (US) Panel only considers vitamin E as the naturally occurring form (*RRR*-) and the other three synthetic 2*R*-stereoisomer forms (*RSR*-, *RRS*-, and *RSS*-) of α-tocopherol. Other naturally occurring forms of vitamin E (β-, γ-, and δ-tocopherols and the tocotrienols) are not considered because, although absorbed, they are poorly recognized by the α-tocopherol transfer protein (α-TTP) and thus more metabolized in the liver (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000).

As we mentioned previously, despite a large number of significant clinical trials conducted with vitamin E supplementation, few studies have shown a link between α-tocopherol intake and beneficial effects against oxidative stress based diseases. This observation can be explained by the fact that vitamin E does not only act as an antioxidant and that the diseases considered in the various studies are multicausal and are not linked solely to oxidative stress. Overall, large randomized clinical trials and some meta-analysis rather underlined a lack of efficacy of vitamin E supplementation in different categories of population to prevent atherosclerosis and cardiovascular disease (Bleys et al. 2006; Lee et al. 2005; Lonn et al. 2005; Ueda and Yasunari 2006), cancer (Graham et al. 1992; Kirsh et al. 2006; Klein et al. 2011; Lee et al. 2005; Leng et al. 2019; Lonn et al. 2005; Lotan et al. 2012; Wu et al. 2002), Alzheimer disease (Farina et al. 2017; Isaac et al. 2008; Kryscio et al. 2017; O'Brien et al. 2017; Rutjes et al. 2018), and age-related macular degeneration (Age-Related Eye Disease Study Research Group 2001; Evans and Lawrenson 2017; Taylor et al. 2002). Long-duration vitamin E supplementation could, however, reduce risk of death from bladder and kidney cancer, but well-designed cohort studies and randomized clinical trials are needed to confirm this observation (Jacobs 2002; Lin et al. 2019; Shen et al. 2015; Wang et al. 2014). Moreover, as already evoked for vitamin C, an increasing fruit and vegetable intake, source of vitamin E, not antioxidant supplement use, could globally reduce the risk of chronic diseases such as total cancer and prevent premature deaths. Optimal intakes for chronic diseases prevention could be about 800 g/day for fruits and vegetables, 225 g/day for whole grains and 15–20 g/day for nuts (Aune 2019; Aune et al. 2018). Vitamin E supplementation at 200 mg/day, to avoid adverse effects, could prevent aging-associated reductions in immune function in elderly human subjects (De la Fuente et al. 2008; Meydani et al. 1997; Pae and Wu 2017; Wu and Meydani 2014). Some studies underlined that vitamin E supplementation could be associated with improvement of immune functions and reduced risk of acquiring upper respiratory infections in some categories of elderly subjects (Hemilä et al. 2004, 2006; Hemila and Kaprio 2011; Meydani et al. 2004). However, these results are controversial (Graat et al. 2002) and could be explained by differences in study design and genetic differences in particular polymorphism (Pae and Wu 2017). The most convincing clinical studies concern the benefices of a vitamin E supplementation in adult patients with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). In adults, vitamin E significantly improved liver function and provides biochemical and histological improvements. However, these positive effects are not observed in pediatric patients and in patients with type 2 diabetes (Amanullah et al. 2019; Anushiravani et al. 2019; Bril et al. 2019; Cankurtaran et al. 2006; Ji et al. 2014;

Pacana and Sanyal 2012; Said and Akhter 2017; Sanyal et al. 2010; Sato et al. 2015; Tsou and Wu 2019). α-tocopherol showed anti-inflammatory effects and it could be interesting in combination with other antioxidants to prevent inflammation-related diseases. Supplementation with α-tocopherol reduce serum C-reactive protein (CRP) levels in patients with cardiovascular diseases (CVD) and having associated risk factors for CVD such as smoking and diabetes. Further studies are needed to confirm this effect in humans (Murphy et al. 2004; Saboori et al. 2015; Schwab et al. 2015; Singh & Jialal 2004).

26.5.5 Applications in Food

Vitamin E is present in several food categories, including animal-based products such as butter, eggs, and fatty fish. However, it is mainly provided by plant species, with the highest contents in oilseeds, nuts and wheat germ. The richest source of vitamin E is wheat germ oil with 144.03 mg/100 g. Table 16 sums up major plant food sources of vitamin E, according to the EFSA Food Composition database (https://www.efsa.europa.eu/en/data/food-composition), selected on the ground of established regulatory thresholds for any food to be considered a source of vitamin E (>1.8 mg/100 g) or rich in vitamin E (>3.6 mg/100 g). The most abundant tocopherol forms found in food are α-tocopherol and γ-tocopherol. Vegetable oils vary in their content of different tocopherol forms. For example, wheat germ and sunflower oil are good sources of α-tocopherol, whereas soybean oil is rich in γ-tocopherol and wheat germ oil is rich in β-tocopherol (EFSA NDA Panel 2015c).

Overall, food preparation procedures have only minor effects on tocopherols and tocotrienols. Cooking and baking have the greatest impact on the loss of tocochromanols (Piironen et al. 1987). Unlike other antioxidant vitamins and some unsaturated fatty acids degraded due to oxidation, fried foods are often a good source of vitamin E (Fillion and Henry 1998). In a study conducted on immature seeds of five cultivars of common bean, the most abundant form of tocopherol found in fresh seeds was γ-form (about 90% of the total), followed by the α- and δ-forms. β-form was not present. The cultivar and the variety, as well as the method of preservation, had no impact on both tocopherol retention and vitamin E activity. Cooking process led to 13–28% decrease in total tocopherols and a 17–31% reduction of vitamin E activity (Słupski and Lisiewska 2013). In the framework of a study conducted on sorghum flours, all processing with dry heat increased the vitamin E content and its retention, unlike wet heat processing (Cardoso et al. 2014). α-tocopherol and γ-tocopherol are also the major tocopherol forms in peanut oil. Oven roasting increases gradually the tocopherol contents in roasted peanut oil (Juhaimi et al. 2018).

26.5.6 Safety: Toxicity and Side Effects

The Panel on Dietary Antioxidants and Related Compounds in the USA and Canada determined a Tolerable Upper Intake Level (UL) for adults at 1000 mg/day (2,325 μmol/day or 1500 UI/day) of any form of supplemental α-tocopherol. This

Table 16 Vitamin E content of plant products, which are at least sources of vitamin E (>1.8 mg/100 g), expressed in α-tocopherol equivalent from vitamin E activities.

Category	Food	Total vitamin E (mg/100 g) (min–max values when available)
Fruit	Avocados	3.23
	Bilberries (European blueberries)	1.87
	Blackberries	2.65
	Blackcurrants juice	1.88
	Dried apricots	3.95
	Dried prunes, raw / process baking / process cooking in water	2.53 / 4.36 / 1.83
	Rose hips and similar	4.14
	Sea buckthorns	3.05
Grains and grain-based products	Cereal bars mixed	4.12
	Cereal bran	2.36
	Corn chips	3.44
	Corn curls	3.54
	Mixed breakfast cereals	2.99
	Muesli plain	2.96
	Multigrain (not only rye-wheat) bread and rolls	2.09
	Processed mixed cereal-based flakes	4.57
	Processed oat-based flakes	1.83
	Processed wheat-based flakes	2.30
	Rusk, wholemeal	3.42
	Wheat bran	2.00
	Wheat bran rolled flakes	4.18
	Wheat germ	**18.02**
	Wheat germs rolled flakes	**22.10**
Nuts and oilseeds	Almonds sweet, raw / process baking / process cooking in water / process steaming	**24.53 / 25.42 / 24.80 / 24.80**
	Bitter almonds	**24.26**
	Cashew nuts, raw / process cooking in water / process steaming	3.15 / 5.54 / 6.02
	Cotton seed oil, edible	**36.79**
	Grape seed oil	**26.82**
	Hazelnuts, raw / process baking / process cooking in water / process steaming	**22.72 / 24.73 / 22.42 / 22.42**
	Linseed oil	**17.50**
	Maize oil, edible	**26.85**
	Olive oil, refined	**10.50**
	Olive oil, virgin or extra-virgin	**10.90**
	Palm kernel oil, edible	**25.60**
	Palm oil/fat	**19.39**
	Peanut butter	7.12

(continued)

Table 16 (continued)

Category	Food	Total vitamin E (mg/100 g) (min–max values when available)
	Peanut oil, edible	**15.16**
	Peanuts, raw / process baking / process cooking in water / process steaming	9.32 / 3.37 / 9.18 / 9.18
	Pecans	7.17
	Pine nuts kernels and similar, raw / process baking / process cooking in water / process steaming	**12.62 / 16.34 / 12.64 / 12.64**
	Pistachios, raw / process baking / process cooking in water / process steaming	4.96 / 4.29 / 4.77 / 4.77
	Poppy seeds	3.04
	Pumpkin seed oil	3.50
	Pumpkin seeds, raw / process baking / process cooking in water / process steaming	4.00 / 3.60 / 3.66 / 3.66
	Rape seed oil	**20.84**
	Safflower seed oil, edible	**43.45**
	Sesame seeds, raw	2.39
	Soya bean oil, refined	**17.50**
	Sunflower seed oil, edible	**63.20**
	Sunflower seeds, raw / process baking / process cooking in water / process steaming	**40.42 / 35.65 / 36.20 / 36.20**
	Table olives ready for consumption	2.79
	Walnut	7.81
	Walnut oil	4.58
	Wheat germ oil	**144.03**
Legumes	Chickpea flour	4.01
	Chickpeas, dried, raw	2.92
Spices and herbs	Allspice fruit	4.50
	Caraway fruit	1.88
	Coriander seed	1.90
	Cumin seed	2.16
	Nutmeg seed	2.72
Starchy vegetables	Potato crisps or sticks	5.08
	Potato starch-based snacks	2.88
	Sweet potatoes, process baking / process steaming	2.42 / 1.97
	Taros	2.40
Vegetables	Angelica (leaves and stems)	2.90
	Asparagus, process baking	2.21
	Aubergines, process frying	3.36
	Chards, process cooking in water	1.81
	Chervil	2.90
	Chili peppers, raw / process cooking in water	2.90 / 3.20

(continued)

Table 16 (continued)

Category	Food	Total vitamin E (mg/100 g) (min–max values when available)
	Chinese cabbages and similar, process baking	5.40
	Common mushrooms, process frying	2.39
	Dandelions	3.42
	Dried mushrooms	4.94
	Garden sorrel	1.90
	Grape leaves, raw / process cooking in water / process steaming	2.00 / 2.23 / 2.23
	Head cabbages, process baking	2.58
	Laurel	2.15
	Mashed vegetable puree	3.44
	Mints	3.14
	Parsley	2.29
	Red mustard leaves	2.90
	Salsifies, process cooking in water / process steaming	2.59 / 1.67
	Sorrel	1.90
	Spinaches, process cooking in water / process steaming	1.88 / 1.87
	Spinaches and similar, process cooking in water / process steaming	1.88 / 1.87
	Spirulina	2.70
	Spring onions, process frying	2.90
	Sun-dried tomatoes	7.46
	Sweet peppers, process frying	2.28
	Thyme	4.38
Various	Cocoa beans (fermented or dried)	4.78
	Cacao mass	2.21
	Cacao powder (sugar free)	4.78
	Coffee beans (roasted)	2.70
	Coffee ground (roasted)	2.70
	Non-fermented tea leaves (green or white tea)	2.10
	Tea leaves and stalks (decaffeinated, fermented)	2.10
	Yeast extract	7.46
	Yeast leavened sweet doughs, process baking	2.39

UL is based on bleeding, an adverse effect observed in animal models at high doses (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds 2000). The Scientific Committee for Food in Europe in 2003 determined a UL for adults, including pregnant and lactating women, at 300 mg α-tocopherol-equivalents per day, based on the determination of a No Observed Adverse Effect Level

(NOAEL) of 540 mg α-TE/day coming from the study conducted by Meydani and collaborators in 1998. The ULs for children were extrapolated and are ranged from 100 mg α-TE/day for children aged 1–3 years old to 260 mg α-TE/day for adolescents (15–17 years) (EFSA NDA Panel 2015c; Meydani et al. 1998). Discordant clinical trials relate to the link between high doses of vitamin E and the increase in all-cause mortality. A meta-analysis underlined that high-dosage ($\geq$ 400 IU/day) vitamin E supplement may increase all-cause mortality (Miller et al. 2005). However, a more recent meta-analysis showed that supplementation with vitamin E had no effect in all-cause mortality at doses up to 5500 IU/day (Abner et al. 2011). Another meta-analysis based on 18 randomized controlled trials in healthy people showed no effect on all-cause mortality with vitamin E supplementation at a dose 23–800 IU/day (Curtis et al. 2014). Some drug interactions can be observed with aspirin, warfarin, tamoxifen and cyclosporine A in the case of high doses vitamin E supplementation (superior to 300 mg/day). Vitamin E may potentiate anti-blood clotting action if taken concomitantly with aspirin and warfarin. It may reduce blood concentration of tamoxifen and cyclosporine A (Podszun and Frank 2014). Because of its anticoagulant properties, taking vitamin E is not recommended in patients with history of stroke or digestive ulcer. For the same reason, vitamin E intake should be stopped 1 month before any surgery

26.5.7 Marketed Products

As α-tocopherol is fat-soluble, it is usually esterified with acetic acid or succinic acid to form α-tocopherol acetate. Indeed, the esters are more chemically stable. After ingestion, they are de-esterified and absorbed as free α-tocopherol.

According to reference website for healthcare professionals in France, α-tocopherol acetate can be marketed orally in some pharmaceutical specialties at a dose of 500 mg per tablet to treat vitamin E deficiency. α-tocopherol acetate can be also marketed in injectable form. In association with ascorbic acid or rutin, α-tocopherol can be used in the care of capillary weaknesses, hemorrhoids, venolymphatic insufficiency, and vascular retinopathies (VIDAL 2016).

α-tocopherol, in general in the form of α-tocopherol acetate dissolved in vegetable oil, can also be found in food supplements, alone, in combination with other vitamins and minerals, or in combination with antioxidant plant extracts. The proposed doses range from 100 to 1000 IU per tablet. Vitamin E can be synthetic or with natural origin from seed oils. γ-tocopherol and tocotrienols are also available in some food supplements.

26.5.8 Patents

A considerable number of patents have been filed on vitamin E since its isolation and identification. The patents relate in particular to optimization of its synthesis, development of methods of extraction from natural sources, novel therapeutic

applications, development of galenic formulations to improve its solubility and to enhance its antioxidant activity for pharmaceutical, agro-food, or cosmetic applications.

26.5.9 Perspectives

Most of the research was focused on α-tocopherol. Additional studies are required to deepen its bioavailability and its physiological role. In-depth investigations on the role of other tocopherols (β-, δ- and γ-tocopherols), tocotrienols, as well as metabolic products, including short- and long-chain metabolites, could bring pathophysiological and pharmacological discoveries. The intake of high doses of vitamin E remains controversial and it seems better to privilege a diet rich in fruits and vegetables rather a vitamin E supplementation.

26.6 Vitamin K1: Phylloquinone

26.6.1 Bioactive Constituents

Vitamin K is a group of lipophilic compounds having in common the 2-methyl-1,4-naphtoquinone (menadione) moiety, which is substituted by a varying number of isoprenoid groups the first of which bears one double bond. The remaining isoprenoid units may vary in number and bear additional unsaturations if they are of microbial origin. In this case they are named menaquinones or vitamin K2. The plant-derived compound is called vitamin K1 (phylloquinone, phytomenadione) and consists of menadione and a linear diterpenoid mono-unsaturated (phytyl) chain (Fig. 34). Synthetic vitamin K may be simplified to the sole menadione moiety, making it possibly more water-soluble. This form is active but does not occur naturally.

Biosynthesis of phylloquinone in plants was described in *Arabidopsis thaliana* after elucidation of the main vitamin K pathway in cyanobacteria and bacteria (Basset et al. 2017). It is depicted in Fig. 35. It is derived from chorismate via its isomer isochorismate by isochorismate synthase (Garcion et al. 2008). Isochorismate is submitted to a series of three enzymatic reactions of a sole protein, encoded by the *PHYLLO* gene, that is comprised of modules that are homologous to separate bacterial proteins in the synthesis of menaquinones, producing *o*-succinyl benzoate (OSM), in chloroplasts (Gross et al. 2006). Cyclization leading to the naphtalenoid

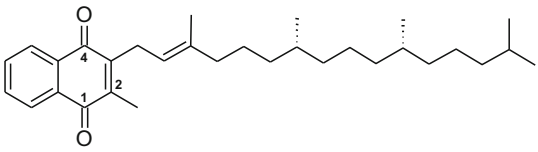


Fig. 34 Chemical structure of phylloquinone

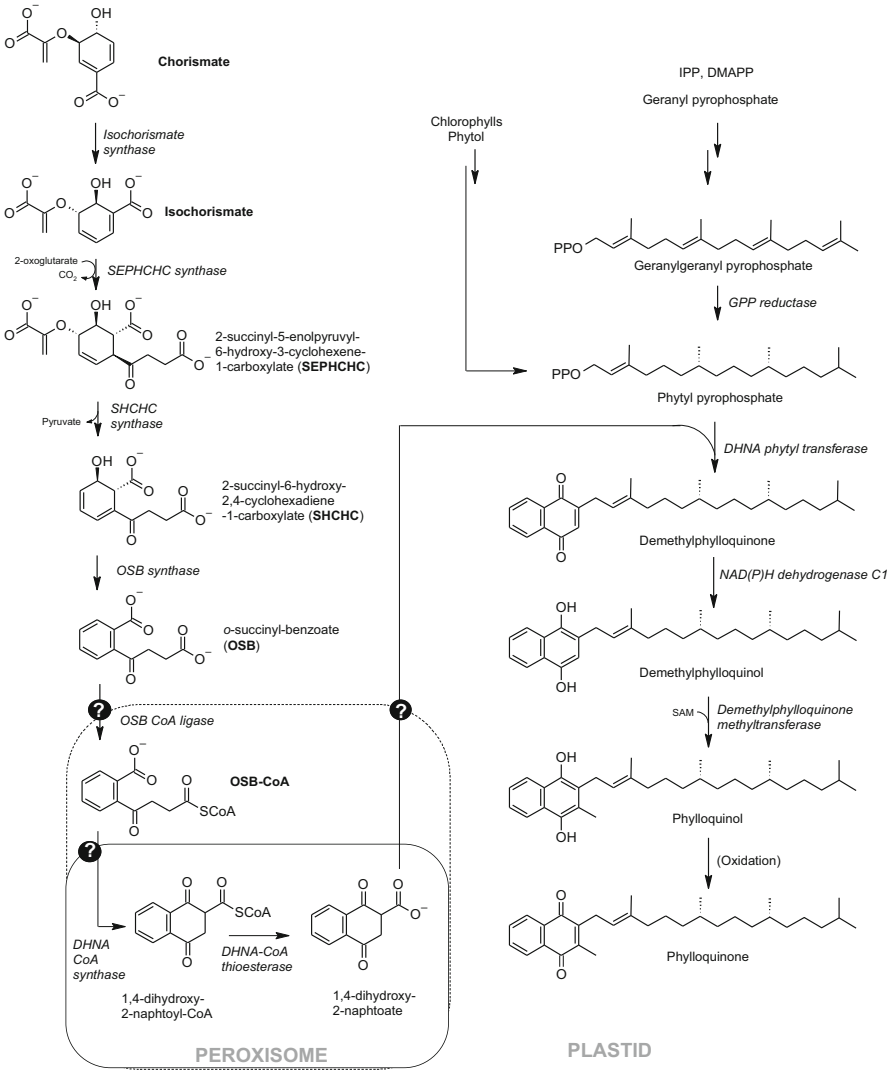


Fig. 35 Biosynthesis of phylloquinone

moiety requires involvement of peroxisomes. OSM activation by coenzyme A may take place also in plastids, but cyclization and subsequent thioester hydrolysis appear to be exclusively peroxisomal. This compartmentalization has been attributed to necessity as the cyanobacterial DHNA thioesterase gene was lost long ago (Reumann 2013). Carrier proteins for transport between cell compartments are not known presently. Attachment of the phytyl chain and decarboxylation lead to demethylphylloquinone (Shimada et al. 2005), which is processed by a methyltransferase, with S-adenosylmethionine as the donor. This last step is

performed on the reduced hydroquinone form of phylloquinone (phylloquinol), enabled by a type II NADPH Dehydrogenase (Fatihi et al. 2015). Because of its high susceptibility to oxidation, phylloquinol is considered to re-oxidize spontaneously to phylloquinone (McCoy et al. 2018).

This biosynthetic pathway exists in prokaryotes (with differences on the phytyl chain leading to menaquinones). Another pathway was described in bacteria (Dairi 2009). This "futalosine" pathway has not been reported in plants to this day.

26.6.2 Bioavailability and Metabolism

26.6.2.1 Bioavailability

Absorption

Humans are not able to synthesize vitamin K which therefore must be provided by the diet via intestinal absorption. Vitamin K1 accounts for about 90% of dietary vitamin K. The absorption of vitamin K1 seems to be relatively similar to that of vitamin E and could implicate the same type of transporters. Like vitamin E, vitamin K1 is released from its food matrix after ingestion and then incorporated into lipid droplets. In the small intestine, phylloquinone is combined by bile salts with other dietary lipids and products of pancreatic lipolysis into mixed micelles which can then reach the intestinal enterocytes (Booth and Suttie 1998; EFSA NDA Panel 2017b; Shearer et al. 1974, 2012; Yamanashi et al. 2017). Like in the case of other lipid-soluble vitamins, it was long assumed that vitamin K was absorbed only by passive diffusion, after dissociation of mixed micelles, through enterocyte apical membrane. But, recent discoveries showed that the absorption of vitamin K is also more complex and could involve the same receptors and transporters as those implicated in the absorption of vitamins A and E, in particular NPC1-like intracellular cholesterol Transporter 1 (NPC1L1), Scavenger Receptor Class B Type 1 (SR-B1), and Cluster of Differentiation 36 (CD36) (Goncalves et al. 2014a, b; Takada et al. 2015; Yamanashi et al. 2017). However, further studies are needed to fully grasp the implication of these different transporters in the absorption of phylloquinone. Absorption of vitamin K1 in healthy adults is very variable and can be ranged from approximately 4% to about 60–64% according to different studies. Indeed, absorption can be impacted by several factors in particular food matrix and fat and fat-soluble micronutrients provided by the diet. Absorption will be increased in presence of butter or oils. Sex and age does not seem to have any effect on absorption of vitamin K, but no data on phylloquinone absorption in infants and children are available. In the state of current knowledge, it is not possible to determine precisely an average absorption of vitamin K from the diet (EFSA NDA Panel 2017b; Jones et al. 2009).

After intestinal absorption, phylloquinone, the predominant form of vitamin K in blood, is mainly incorporated in intestinal epithelial cells into nascent chylomicrons and secreted into the systemic circulation from chylomicrons along the lymphatic pathway. However, even if some complementary studies are required, the ATP-

Binding Cassette Transporter A1 could be probably involved in the secretion of vitamin K into portal blood with intestinal HDL, as for vitamin E (Yamanashi et al. 2017). In blood, no specific carrier protein does not appear to have been identified. The main transporters are triglyceride-rich lipoproteins (TRL), with about 75% to 90% of plasma phylloquinone. The other transporters are chylomicrons remnants and very low-density lipoproteins (VLDL), and to a lesser extent low- and high-density lipoproteins (EFSA NDA Panel 2017b; Erkkilä et al. 2004). Vitamin K1 is rapidly absorbed. Its peak in plasma appears about 4–10 h after ingestion. Depending on studies, the plasma half-life of phylloquinone ranged between 0.22 h and 8.80 h. Vitamin K1 has a rapid removal rate from the circulation (Fig. 36) (EFSA NDA Panel 2017b; Halder et al. 2019).

Among menaquinones (vitamin K2), MK-7 exhibited greatest bioavailability and is rapidly absorbed such as vitamin K1 (Halder et al. 2019).

Distribution

The liver is the main organ that accrues absorbed phylloquinone transported in chylomicrons. Vitamin K1 enters the liver through the endocytosis of chylomicron remnants and involves different apolipoproteins and high-affinity lipopoprotein receptors. ApoE on the surface of chylomicrons is essential for transport and cellular internalization of vitamin K in liver. It facilitates high-affinity binding of lipoproteins to the LDLR (LDL-receptors) and other members of LDLR family such as LRP (LDL receptor-related protein) on the surface of hepatocytes. ApoB-48 is also implicated. Chylomicrons are then taken up by hepatocytes by receptor-mediated endocytosis. The major portion of vitamin K1 is stored in the liver. The rest will join the vitamin K2 and will be exported to other tissues by incorporation in VLDL, mainly bearing surface Apo-E and ApoB-100. In blood, VLDL undergoes dilapidation to form VLDL remnants (IDL) and then LDL (Akbari and Rasouli-Ghahroudi 2018; Shearer et al. 2012). The mean vitamin K1 concentration in the liver ranged from about 3 and 34 ng/g and is lower than the mean vitamin K2 concentration. The mean vitamin K1 percentage in the total content of vitamin K of the human liver is large, ranged from 2.4% to 74%, due to several reasons: conversion of phylloquinone to menadione and then to MK-4 by cellular alkylation, degradation of phylloquinone during tissue handling and storage, variability in phylloquinone intake (EFSA NDA Panel 2017b; Institute of Medicine (U.S.) and Panel on Micronutrients 2001; Shearer et al. 2012). Skeletal muscle contains little phylloquinone, but significant concentrations are found in the heart and some other extrahepatic tissues such as bone. Osteoblasts are also able to internalize vitamin K1 from various lipoproteins fractions, directly from chylomicron remnants or from LDL coming from the catabolism of VLDL previously formed in the liver. ApoE and ApoB-48 on the surface of chylomicrons, as well as ApoB-100 on the surface of LDL, are essential for transport and cellular internalization of vitamin K in bone. ApoB-100 facilitates binding of LDL to the LDLR and APOE and ApoB-48 facilitates binding of chylomicrons remnants to LDL receptor-related protein 1 (LRP1) on the surface of osteoblasts (EFSA NDA Panel 2017b; Shearer et al. 2012). Otherwise, during pregnancy, small quantities of phylloquinone pass through the placental barrier. The

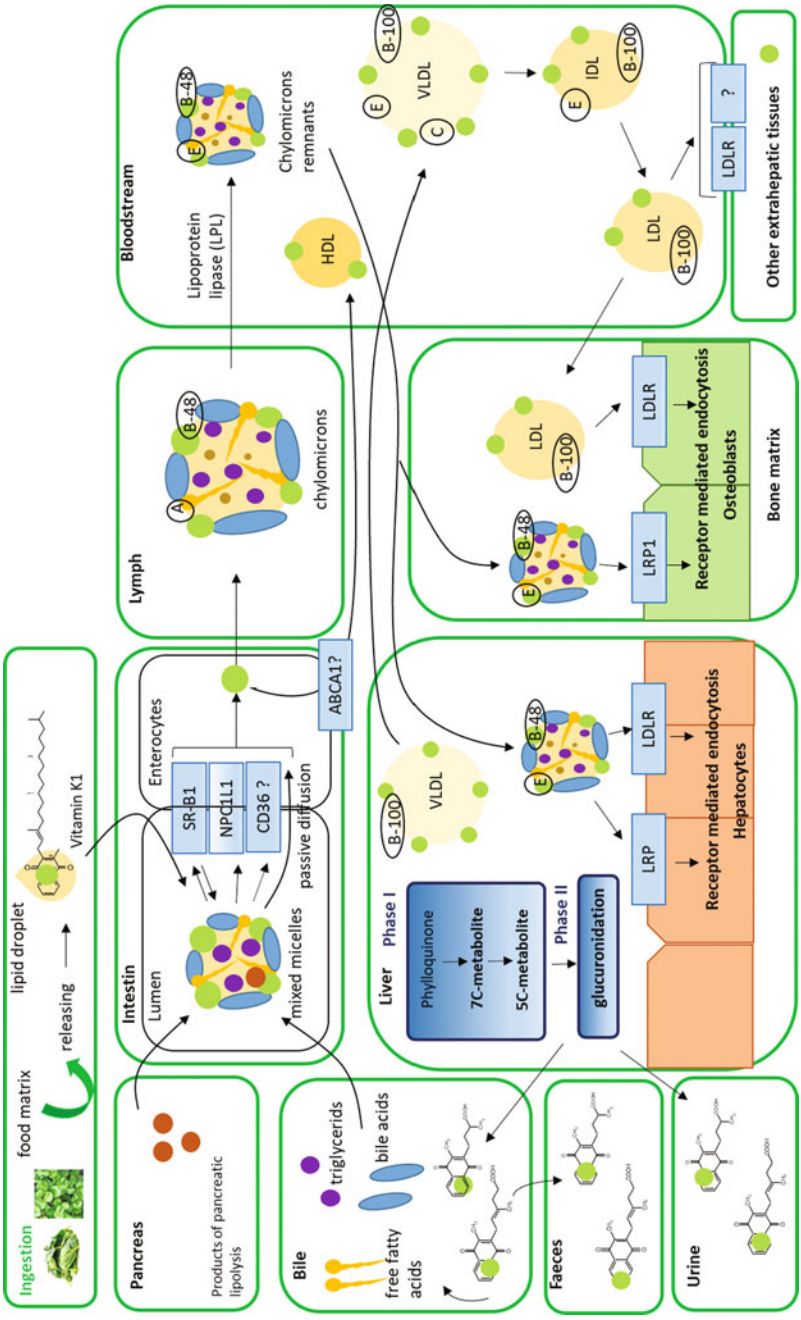


Fig. 36 (continued)

turnover of phylloquinone in the tissue is slower than in plasma with a tissue half-life in the range of 1.8–215 h (Fig. 36) (EFSA NDA Panel 2017b).

26.6.2.2 Metabolism

In the liver, phylloquinone is catabolized according to the same degradative pathway than menaquinones. In phase I, phylloquinone is metabolized by an initial ω-hydroxylation, followed by β-oxidation to provide two major metabolites with shorter side chains: five carbon vitamin K metabolite (5C-metabolite) named 2-methyl-3-(3′,3′-carboxymethylpropyl)-1,4-naphtoquinone and seven carbon vitamin K metabolite (7C-metabolite) named 2-methyl-3-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-naphtoquinone (Fig. 37). In phase II, these metabolites are conjugated with glucuronic acid and excreted in the bile and the urine. In the case of patients treated with warfarin, the ingestion of a large dose of phylloquinone (400 mg) led to the formation of a third aglycone metabolite in urines, a ten-carbon vitamin K metabolite (10C-metabolite) named 2-methyl-3-(7′-carboxy-3′,7′-dimethyl-2′-heptenyl)-1,4-naphtoquinone (EFSA NDA Panel 2017b; McBurney et al. 1980; Shearer and Newman 2014). Overall, phylloquinone metabolites seem to be eliminated in an equivalent manner in the feces via the bile and in urine. The 5C-metabolite is the main urinary metabolite. However, with a high oral intake of vitamin K (1 mg), non-absorbed phylloquinone and phylloquinone metabolites are eliminated in larger amount in feces (up to 60%) (EFSA NDA Panel 2017b).

26.6.3 Bioactivities

26.6.3.1 General Role (Animal Studies)

All forms of vitamin K have a role of enzymatic co-factor required for γ-carboxylation of glutamate residues, a post-translational modification of proteins. This role relies on vitamin K activation and regeneration following a pattern that is classically known as the "vitamin K cycle" (Fig. 38).

Vitamin K is used in its reduced hydroquinone form by γ-glutamyl carboxylase (GGC) (Berkner et al. 1992) in the endoplasmic reticulum, together with CO_2 and H_2O, a reaction that converts vitamin K hydroquinone into a 2,3-epoxide derivative. GGC is thought to activate vitamin K by turning it into its alkoxide derivative, a stronger base with the capacity to abstract the γ proton, enabling carboxylation at this position, a principle labeled as the "base amplification model" (Dowd et al. 1995).

Fig. 36 Absorption, distribution and metabolism of vitamin K1. Adapted from (Shearer et al. 2012; Yamanashi et al. 2017). A, apolipoprotein A; ABCA1, ATP-binding cassette transporter; B-48, apolipoprotein B48; B-100, apolipoprotein B100; E, apolipoprotein E; αHDL, high density lipoproteins; NPC1L1, Niemann-Pick C1-like intracellular cholesterol Transporter 1; SR-B1: Scavenger Receptor Class B Type 1; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; LDLR, LDL receptor; LRP, LDL receptor-related proteins; LRP1, LDL receptor-related protein 1; VLDL, very low density lipoproteins.

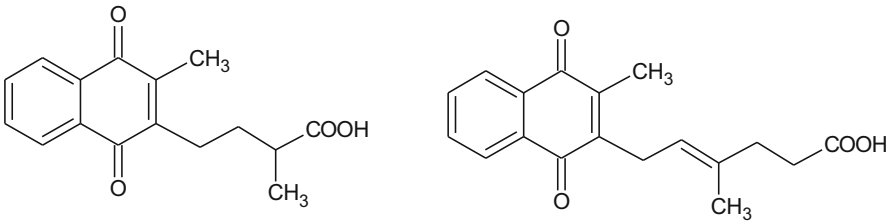


Fig. 37 2-methyl-3-(3′,3′-carboxymethylpropyl)-1,4-naphtoquinone (5C-metabolite) and 2-methyl-3-(5′-carboxy 3′-methyl-2′-pentenyl)-1,4-naphtoquinone (7C-metabolite), major metabolites of vitamin K1 in the liver

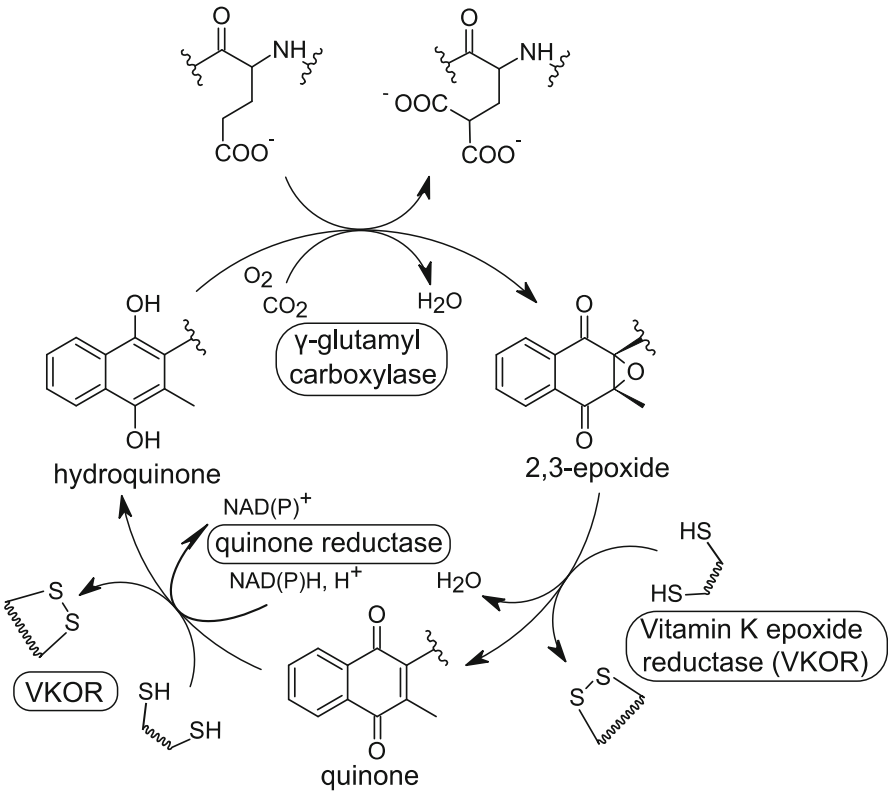


Fig. 38 Vitamin K cycle

The deprotonation of vitamin K hydroquinone was first attributed to a cysteine residue (Furie et al. 1999); more recently, the critical weak alkaline grouping was shown to be the side-chain amine in Lys218 (Rishavy et al. 2006) (Fig. 39).

Vitamin K epoxide reductase (VKOR), an enzyme that uses thiol groups as the reducing agent (Silverman and Nandi 1988), operates the opening of the epoxide and

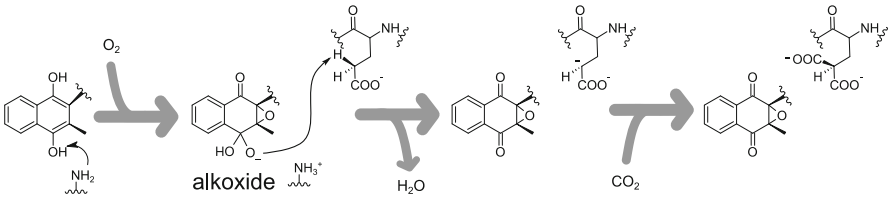


Fig. 39 Proposed mechanism of γ-glutamyl carboxylation by GGC (Furie et al. 1999; Rishavy et al. 2006)

conversion to the quinone derivative. VKOR exists as paralogs VKORC1 and VKORC1L1 that both contribute to vitamin K regeneration (Lacombe and Ferron 2018).

The reduction into the hydroquinone form can be performed by VKOR as well (Gardill and Suttie 1990), following a mechanism that can be reasonably thought to be similar to that of epoxide reduction, as depicted by Preusch and Smalley (1990) (Fig. 40). Nevertheless, additional vitamin K reductase activities have been detected (Ingram et al. 2013). Two FAD-associated quinone reductases were considered: NQO1 (NAD(P)H quinone reductase 1 = DT-diaphorase), which is upregulated in some cancers and known to be responsible for mitomycin C activating bioreduction uses NADH or NADPH as reductant; and NQO2 (NRH quinone oxidoreductase 2), which has only been characterized by its ability to use dihydronicotinamide riboside (NRH), a non-physiological compound (Megarity et al. 2014). NQO1 was found to have a moderate effect on vitamin K hydroquinone production in mice (Ingram et al. 2013) and shown to be more active on synthetic vitamin K3 than on natural K1 or K2. NQO2 may enhance menadione toxicity by operating only one electron reduction to produce a semiquinone that reoxidizes readily to the quinone form. Its effective role for natural vitamin K reduction is not established either (Gong et al. 2008). Nevertheless, a NAD(P)H-dependent vitamin K reductase different from NQO1 is thought to exist, at least in mice (Ingram et al. 2013).

Proteins affected by glutamyl γ-carboxylation are involved in blood coagulation, bone metabolism, and vascular biology. Of course, hypocoagulation, the most obvious trouble linked to vitamin k deficiency in chick experimental restricted diets was also the first identified and led to the identification of this *Koagulationsvitamin* in 1929 by Danish researcher Henrik Dam (carpenter 2003b), who received the Nobel Prize in Physiology and Medicine in 1943 (Dam 1946). As bleeding induced by sweet clover consumption in cattle was the starting point for the identification of dicoumarol as a vitamin K antagonist and the development of therapeutic agents such as warfarin, abnormalities in bovine blood samples were a key material for identification of vitamin K mechanism of action (Nelsestuen and Suttie 1972; Stenflo et al. 1974) as well as of proteins that required it for their maturation, for example, prothrombin (Ganrot and Nilehn 1968).

The role of vitamin K in bone was also primarily discovered in chick bone microsomes (Lian and Friedman 1978).

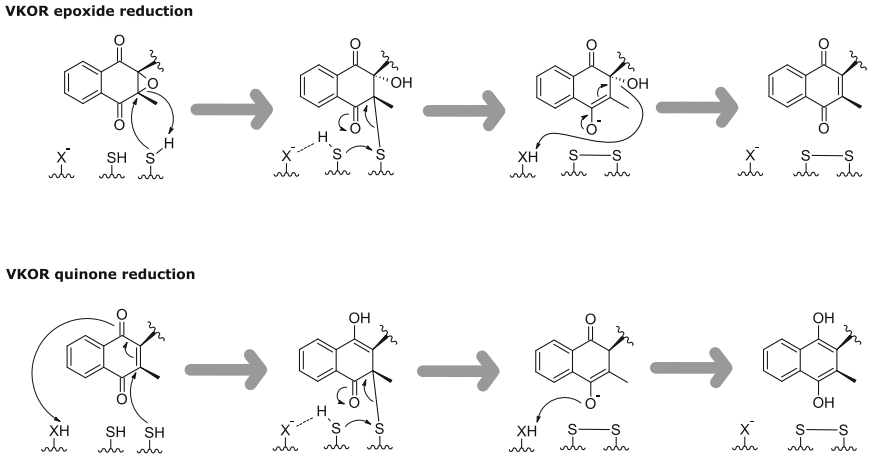


Fig. 40 Proposed mechanisms for VKOR reduction of vitamin K epoxide (up) and quinone (down), adapted from Preusch and Smalley (1990).

26.6.4 Benefits (Human Studies)

A variety of proteins require vitamin K for their maturation. Most of them to this date are coagulation factors: Factors II (prothrombin), VII, IX, X, proteins C, S, Z and Gas6 (Wallin et al. 2008). So-called Gla domains are located in the N-terminal region of these proteins and can only be γ-carboxylated as propeptides: a signal peptide is required for processing and is later hydrolyzed by furin for secretion of the mature molecule. This is exemplified by the sequence of factor VII/VIIa which requires γ-carboxylation among other post-translational modifications, as depicted in Fig. 41. γ-carboxylation enables Ca^{2+} chelation and interaction with phospholipids at the site of injury, making in most cases their activation possible (Furie and Furie 1992).

In Europe, EFSA NDA Panel recognized that "Vitamin K contributes to normal blood clotting," but stated that no effect may be observed for prolonged deliberately restricted diet in humans (EFSA NDA Panel 2009e).

Apart from hemostasis, the most studied vitamin K-dependent proteins are involved in bone metabolism:

- Osteocalcin was the first to be discovered and the identification its γ-carboxylated nature preceded that of vitamin K metabolism in the bone (Price et al. 1976). Osteocalcin is a biomarker of osteoblastic activity and contributes to the regulation of bone mineralization but does not raise bone mass (Diegel et al. 2020; Moriishi et al. 2020). It has been postulated that its binding to calcium in bone may be a mode of modulating the hormonal effects of its decarboxylated form on energy metabolism (Lee et al. 2007) and stress (Meyer Berger et al. 2019) but this was not confirmed outside the research team that formulated this theory (Manolagas 2020).

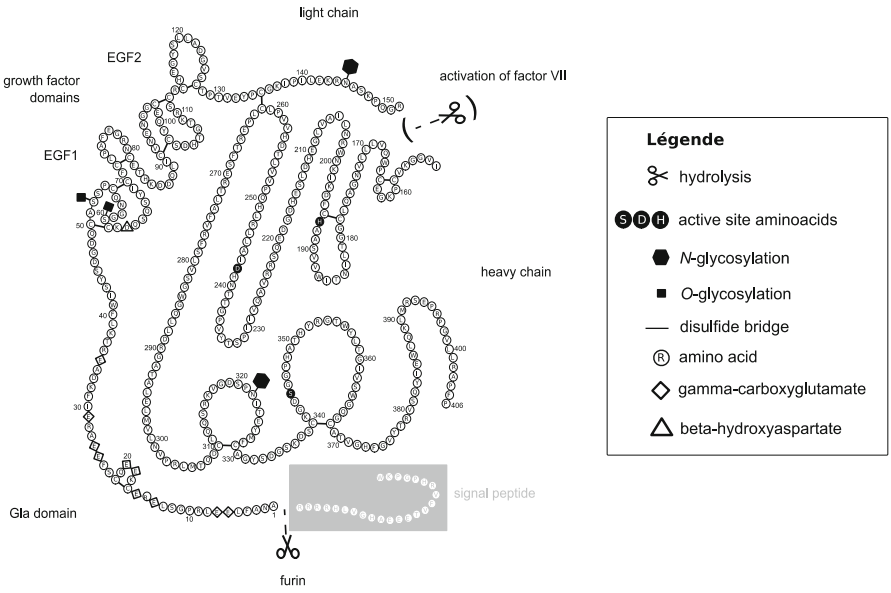


Fig. 41 Sequence of factor VIIa, with Gla domain and signal peptide shown (Hennebelle 2020)

- Matrix Gla-protein (MGP) is an inhibitor of tissue calcifications that is secreted by chondrocytes among other cell types (Cancela et al. 2014).

Other proteins possibly involved in bone metabolism requiring vitamin K activation were identified: growth arrest-specific 6 protein (Gas6), Gla-rich protein (GRP), and periostin (De Caterina et al. 2020).

Low vitamin K status has been correlated with lower bone mineral density. Vitamin K supplementation led to mixed results, with favorable outcome predominantly in Japanese populations (Bügel 2008; EFSA NDA Panel 2009e). Negative effect of vitamin K antagonists has remained controversial for a long time, but comparison with direct oral anticoagulants seems to support it, and thus, the protective role of vitamin K on bones (De Caterina et al. 2020).

In Europe, the health claim "Vitamin K contributes to the maintenance of normal bones" has been authorized (EFSA NDA Panel 2009e).

The incomplete knowledge of numerous vitamin K-dependent proteins and their possible implication in blood vessel calcification and cell proliferation has led to investigations on the possible benefit of vitamin K supplementation in a broader spectrum of diseases. As regards cardiovascular disease, higher vitamin K consumption may be correlated with a lower risk of coronary heart disease (Chen et al. 2019a). Positive findings regarding the risk of various cardiovascular events were obtained using desphospho-uncarboxylated matrix γ-carboxyglutamate protein (dp-ucMGP) as a biomarker, but its value for the estimation of vitamin K status is uncertain, all the more because circulating osteocalcin level did not provide such

Table 17 Dietary reference values of vitamin K from EFSA (based on phylloquinone only), expressed in µg/day (EFSA NDA Panel 2017b).

Infants	
0–6 months	–
7–11 months	10
Children	
1–3 years	12
4–6 years	20
7–10 years	30
11–12 years	45
Adolescents	
13–14 years	45
15–17 years	65
Adults	
18–39 years	70
Pregnancy	70
Breastfeeding	70

correlation. Moreover, low circulating phylloquinone levels were associated with an increased risk of all-cause mortality, but not of cardiovascular disease (Shea et al. 2020). Previous studies regarding cardiovascular health and vitamin K status were reviewed by EFSA NDA Panel (2017b).

A possible interest of vitamin K supplementation has been suggested in cystic fibrosis, as malabsorption of this vitamin is possible. It is not clear at present whether supplementation provides benefits (Jagannath et al. 2020).

Adequate intakes (AIs) for children and adults were determined by European Food Safety Authority. Due to a lack of evidence on intake, absorption, biological role, and content in the body and organs of menaquinones, AIs were determined only for phylloquinone. 1 µg/kg body weight per day was established for all age and sex population groups (Table 17).

Vitamin K deficiency is rare in Humans. Some possible causes are:

- Reduced formation of gut microflora menaquinones, for instance during prolonged antibiotic therapy (Liu et al. 2019), particularly when dietary supply cannot replace it, because of restricted diet (Itagaki and Hagino 2019) or in neonates (Aziz and Patil 2015).
- Reduced intake in the context of vomiting, possibly observed in hyperemesis gravidarum (Baba et al. 2016).
- Decreased absorption because of reduced intestinal capacity, which may be observed in bariatric surgery. Bleeding disorders are suggestive of vitamin K deficiency in the general context of lipid-soluble deficiency; persistent diarrhea may hinder dietary vitamin K absorption and curative antibiotic use may aggravate it (Elalfy et al. 2018).
- Drug therapy or intoxication by vitamin K antagonists such as warfarin.

26.6.5 Applications in Food

Phylloquinone, present in all photosynthetic plant species, is the major source of vitamin K in the human diet. The richest sources of vitamin K1 are dark green leafy vegetables (spinaches, lettuces, purslanes, watercresses) and cabbages (broccoli, Brussel sprouts, kales). Phylloquinone can be also found in some legumes and herbs. For example, thyme contains high level of vitamin K. Table 18 sums up major plant food sources of vitamin K1, according to the EFSA Food Composition database (https://www.efsa.europa.eu/en/data/food-composition), selected on the ground of established regulatory thresholds for any food

Table 18 Phylloquinone content of plant products which are at least sources of vitamin K1

Category	Food	Total vitamin K (μg/100 g)
Fruit	Avocados	14.00
	Bilberries (European blueberries)	12.00
	Blackcurrants	30.00
	Blueberries	12.00
	Dehydrated/powdered vegetable juice	57.22
	Dried apples	18.00
	Dried apricots	18.00
	Dried pears	19.00
	Dried prunes / process baking / process cooking in water / process steaming	39.00 / 40.00 / 17.00 / 16.00
	Kiwi fruits (green, red, yellow)	25.00
	Redcurrants	11.00
	Rose hips and similar / jam	92.00 / 17.00
	Wine grapes	13.71
Grains and grain-based products	Biscuits, oat meal	23.00
	Cereal bars mixed	16.00
	Cereal bars plain	16.00
	Cereal and cereal like-grains not separately listed	23.00
	Maize semolina, process baking / process cooking in water / process steaming	15.00 / 15.00 / 15.00
	Oat porridge, ingred tap water	16.00
	Pasta flavored and/or colored-deprecated	73.00
	Processed mixed cereal-based flakes	63.00
	Processed wheat-based flakes	12.00
	Wheat germs rolled flakes	17.00
	Wheat semolina porridge, ingred tap water	15.00
Nuts and oilseeds	Hazelnuts/ process baking	11.00 / 11.00
	Pistachios, raw / process cooking in water / process steaming	59.00 / 54.00 / 54.00
	Poppy seeds, process baking	18.00
	Sunflower seeds, raw / process baking	24.04 / 19.82

(continued)

Table 18 (continued)

Category	Food	Total vitamin K (μg/100 g)
Legumes	Beans (dry) and similar	170.00
	Borlotti or other common beans (dry)	170.00
	Canned or jarred chickpea	200.00
	Canned or jarred common beans	70.00
	Canned or jarred peas	31.12
	Chickpeas (dry) / process cooking in water	264.00 / 13.70
	Garden peas (dry)	81.00
	Lentils (dry)	22.00
	Lentil sprouts, process steaming	33.00
	Lentils, fresh / process baking	22.00 / 15.00
	Lentils (without pods)	22.00
	Mung beans (dry) / process cooking in water / process steaming	147.14 / 85.00 / 85.00
	Peas (without pods) and similar / process baking / process cooking in water / process steaming	42.80 / 39.00 / 23.00 / 37.00
	Peas (with pods) and similar / process cooking in water / process frying / process steaming	28.00 / 28.00 / 28.00 / 28.00
	Soya proteins	54.50
Spices, herbs and sauces	Aniseed myrtle	310.00
	Basil	310.00
	Ginger roots	12.83
	Laurel	310.00
	Pesto	187.04
	Tofu	16.00
	Soy sauce	58.00
	Tomato-containing cooking sauces	12.00
	Thyme	617.22
	Vegetables-based cooked sauces	11.00
Vegetables	Alfalfa sprouts, raw / process cooking in water / process steaming	30.50 / 30.50 / 30.50
	Asparagus, raw / process baking / process cooking in water/ process steaming	39.00 / 43.00 / 39.00 / 41.00
	Aubergines, process frying	16.40
	Beetroots, process baking	100.00
	Broccoli and similar, raw / process baking / process cooking in water/ process frying / process steaming	173.29 / 149.25 / 149.25 / 188.00 / 149.25
	Brussels sprouts, raw / process baking / process cooking in water	162.57 / 140.29 / 140.29
	Canned mushrooms, process baking	18.00
	Carrots, raw / process baking / process cooking in water/ process steaming / juice	15.67 / 16.00 / 16.00 / 15.00 / 16.00
	Cauliflowers, raw / process baking / process cooking in water/ process steaming	28.86 / 26.86 / 26.86 / 26.86

(continued)

Table 18 (continued)

Category	Food	Total vitamin K (μg/100 g)
	Celeriacs, raw / process baking / process cooking in water/ process steaming	41.00 / 45.00 / 40.00 / 43.00
	Celeries, raw / process cooking in water / juice	29.00 / 32.00 / 43.00
	Chards, raw / process baking / process cooking in water/ process steaming	255.00 / 255.00 /255.00 / 255.00
	Chinese cabbages and similar, raw / process baking / process cooking in water	80.00 / 89.00 / 89.00
	Chives	380.00
	Chives and similar	380.00
	Common mushrooms, raw / process baking / process cooking in water/ process steaming	14.00 / 17.00 / 16.00 / 17.00
	Courgettes and similar, process cooking in water	11.00
	Cresses	560.00
	Cucumbers	13.33
	Curly kales, raw / process cooking in water/ process steaming	817.00 / 719.00 / 719.00
	Dried mushrooms, raw / process cooking in water/ process steaming	164.00 / 15.00 / 15.00
	Dried vegetables	164.00
	Escaroles	231.00
	Escaroles and similar	231.00
	Fennel leaves	790.00
	Gherkins	13.00
	Grape leaves, raw / process cooking in water / process steaming	109.00 / 121.00 / 121.00
	Head cabbages / process baking / process cooking in water	59.00 / 62.64 / 62.64
	Head cabbages and similar / process baking / process cooking in water	59.00 / 62.64 / 62.64
	Kales and similar / process cooking in water / process steaming	817.00 / 719.00 / 719.00
	Leeks / process baking / process cooking in water / process steaming	48.17 / 52.00 / 47.00 / 49.00
	Lettuces (generic)	126.67
	Mixed-vegetable juice	17.71
	Multi-vegetables juice	18.50
	Nasturtium flowers and leaves	250.00
	Palm hearts, process steaming	33.00
	Parsley	488.75
	Purslanes	381.00
	Purslanes and similar	381.00
	Red mustard leaves	560.00
	Rhubarbs / process cooking in water / process steaming	11.00 / 12.00 / 12.00

(continued)

Table 18 (continued)

Category	Food	Total vitamin K (μg/100 g)
	Roman rocket	250.00
	Roman rocket and similar	250.00
	Sauerkraut / process baking / process cooking in water / process steaming	25.00 / 28.00 / 28.00/ 28.00
	Spinaches / process baking / process cooking in water / process steaming	362.50 / 420.00 / 426.00/ 401.00
	Spinaches and similar / process baking / process cooking in water / process steaming	381.00 / 420.00 / 426.00/ 401.00
	Sun-dried tomatoes	23.60
	Tomato-leafy vegetables juice	14.80
	Vegetable juice concentrate	24.00
	Watercresses	309.48
	Watercresses and similar	250.00
Various	Non-fermented tea leaves (green or white tea)	262.00
	Tea leaves and stalks decaffeinated	262.00
	Teal leaves and stalks, fermented	262.00

to be considered a source of vitamin K (>11 μg/100 g) or rich in vitamin K (>22 μg/100 g). Menaquinones (vitamin K2), not described in this chapter, are found in animal products such as liver products, meat, cheese, and eggs (EFSA NDA Panel 2017b).

No particular trend was noted as regards seasonal variation of phylloquinone in vegetables. The cooking process does not affect phylloquinone and may even significantly enhance available amount by disruption of cell walls and release from chloroplasts (Damon et al. 2005).

Some vegetable oils provide significant phylloquinone amounts, for example, soy (362 μg/100 g), olive (60.2 μg/100 g), colza (71.3 μg/100 g) and cottonseed (24.3 μg/100 g) oils (ANSES 2017; Peterson et al. 2002).

26.6.6 Safety: Toxicity and Side Effects

There has been no report of any negative effect of supranutritional doses of any form of vitamin K in animals or humans. Studies were mostly performed with phylloquinone (EFSA SCF and NDA Panel 2006). Increased intakes of all forms of vitamin K counter the action of vitamin K antagonists, thus reducing their benefit in cardiovascular disease. Thus their consumption should be controlled or meal composition should be as regular as possible in such cases.

26.6.7 Marketed Products

Vitamin K1 (often labeled as phytomenadione) is used as a pharmaceutical ingredient, for supplementation in cases of deficiency cited above. Its origin seems to be synthetic as it may contain menadione, which is not a natural precursor of phylloquinone (see Fig. 35). The product may contain up to 15% of the (Z) isomer according to the European Pharmacopoeia (2020).

Phylloquinone and menaquinones (in particular MK-6 and MK-7) are added in some foods and food supplements (EFSA NDA Panel 2017b).

26.6.8 Patents

Patents have been dedicated to the preparation of synthetic phytomenadione (CN104744230A), and specially preparation of the pure (*E*) isomer (WO2016038626A1, EP2868658A1). Its cosmetic use has also been considered, as an energizing ingredient (US20070243146A1).

26.6.9 Perspectives

Vitamin K1 has been the most studied vitamer of vitamin K, because of its abundance in the diet and greater simplicity. In recent years, vitamin K2 has raised great interest because of possible better bioavailability and efficiency (EFSA NDA Panel 2017b), but also probably because it may be produced biotechnologically, owing to its microbial origin. Such production is feasible with phylloquinone (Tarento et al. 2018, 2019) making it a competitive candidate for vitamin K formulation, as interest for effects not related to coagulation disorders seems to grow.

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164), maintenance of normal skin (ID 164), maintenance of normal nails (ID 164), maintenance of normal cardiac function (ID 166), maintenance of normal vision by protection of the lens of the eye (ID 167), contribution to normal cognitive function (ID 182, 183), regeneration of the reduced form of vitamin C (ID 203), maintenance of normal blood circulation (ID 216) and maintenance of normal a scalp (ID 2873) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 8:1816

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Curcumin in Food

27

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Contents

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Abstract

Curcumin is the major bioactive constituent of turmeric (*Curcuma longa* L., Zingiberaceae) rhizome. Turmeric has been used as spice and dye but also in the traditional Chinese and Indian medicine for the treatment of respiratory ailments, wounds, gastrointestinal complaints, hepatic disorders, and cardiovascular disease. Numerous studies reported curcumin as an important pleiotropic agent, with anti-inflammatory, wound healing, antioxidant, hypoglycemic, cardioprotective, neuroprotective, hepatoprotective, antimicrobial, chemopreventive, and anticancer activities. These effects have been demonstrated both in vitro and in experimental animal models, thus paving the way for various clinical trials. The latter investigated different curcumin-based formulations, including highly bioavailable formulations showing their efficacy in central nervous system, cardiovascular, gastrointestinal, liver, and metabolic diseases. Benefits in certain malignant diseases were also reported. Curcumin is marketed worldwide as dietary supplement and herbal medicine, as cosmetic product, or as food additive. Curcumin possesses a good safety profile. Chemical features of curcumin, bioactivity and mechanisms of activity, safety profile, patents, and marketed products are summarized in this chapter. As literature on the topic of curcumin has known an exponential growth, reports cited in this chapter should be considered as illustrative rather than comprehensive.

Keywords

Curcumin · *Curcuma longa* · Turmeric · Bioavailability · Anti-inflammatory · Neuroprotective · Chemopreventive · Curcumin formulations

27.1 Introduction

Curcumin represents the major bioactive constituent-derived turmeric (*Curcuma longa* L., Zingiberaceae) rhizome, accounting from 0.58% to 3.14% of its dry weight (Aggarwal et al. 2007). Turmeric has a long record of use as spice and dye; also, it has been used as a traditional Chinese and Indian medicine since immemorial times for the treatment of cough and respiratory ailments, wounds of different etiologies, gastrointestinal complaints, hepatic disorders, and cardiovascular disease. In combination with coriander, cumin, fenugreek and chili peppers, turmeric forms the curry powder, a dietary spice used worldwide (Sarker and Nahar 2007; Esatbeyoglu et al. 2012).

The revival of natural and complementary therapies in Western medicine has drawn the attention of the scientific community to this remedy. Extensive research over the five last decades has shown that curcumin is an important pleiotropic agent, with anti-inflammatory, wound healing, antioxidant, hypoglycemic,

cardioprotective, neuroprotective, hepatoprotective, antimicrobial, chemopreventive, and anticancer activities (Prasad et al. 2014; Kunnumakkara et al. 2017).

These effects have been demonstrated both in vitro, in cultured cells, and in experimental animal models, thus paving the way for various clinical trials. Studies documenting the chemical features of curcumin, its bioactivity and mechanisms of action, safety profile, patents, and marketed products are summarized in this chapter. As literature on the topic of curcumin has known an exponential growth, reports cited in this chapter should be considered as illustrative rather than comprehensive.

Due to its numerous properties, curcumin is being marketed worldwide, either as dietary supplement and herbal medicine, cosmetic products, or as food additive. Curcumin possesses a good safety profile. This was also confirmed by Food and Drug Administration (FDA), who classified curcumin as *generally recognized as safe* (GRAS) by approving the commercialization of a curcuminoid preparation containing curcumin 75–81%, bisdemethoxycurcumin 2.2–6.5%, and demethoxycurcumin 15–22% (Mahran et al. 2017). More, European Food Safety Authority (EFSA) reports an allowable daily intake value for curcumin of 0–3 mg/kg body weight (b.w.) (EFSA 2014).

27.2 Chemical Structure

In the early nineteenth century, curcumin was isolated as a yellow powder from the dried rhizome of *Curcuma longa*. Chemically, curcumin belongs to the class of diarylheptanoids, being a *bis*-α,β-unsaturated β-diketone. It is also known as diferuloylmethane or 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. The molecular formula of curcumin is $C_{21}H_{20}O_6$, and it has a molecular weight of 368.39 g/mol^{-1} (Anand et al. 2007).

Chemically, curcumin is an unstable molecule, being sensitive to alkaline pH, oxygen, and irradiation with ultraviolet and visible light. Curcumin presents a specific keto-enol tautomerism (Fig. 1), whose equilibrium is dependent upon the pH value and polarity of the solvent. The ketone form is metabolically targeted for reduction and conjugation: curcumin is more susceptible to degradation and reduction at the diketo moiety, and conjugation is mediated by the hydroxyl groups (Priyadarsini 2014; Stanić 2017).

Curcumin is an unstable molecule, being degraded mostly via an autoxidative process to bicyclopentadione (Fig. 2) as major product and vanillin and ferulic acid in a lesser extent (Gordon et al. 2015). Interestingly, bicyclopentadione is also formed by catalytic oxygenation through cyclooxygenases and lipoxygenases, thus being not only a degradation product but also a metabolite (Schneider et al. 2015).

27.3 Bioavailability and Metabolism

Curcumin bioavailability and metabolism have been investigated in numerous animal and human studies. After oral administration, curcumin undergoes limited absorption in the small intestine, followed by metabolization in the intestine and

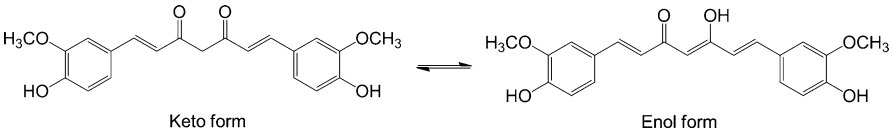
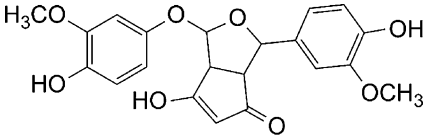


Fig. 1 Keto-enol tautomerism of curcumin

Fig. 2 Bicyclopentadione



liver, and rapid elimination through feces and in a lesser extent through urine (Esatbeyoglu et al. 2012). The low uptake of orally administered curcumin was confirmed in animal models, when oral bioavailability of the polyphenol was found to be lower than 1% (Asai and Miyazawa 2000; Yang et al. 2007).

Human studies also indicate a low absorption of curcumin in the gastrointestinal tract, as after oral administration only minute amounts of curcumin reach peripheral blood. In a clinical study enrolling 15 patients with advanced colorectal cancer which were administered oral doses of 36 up to 180 mg, no detectable plasma levels of curcumin were determined (Sharma et al. 2001). Even after ingestion of high doses of curcumin, up to 8 g, within 1 h after administration, curcumin yielded peak serum concentrations of only 0.5–2 μM (Cheng et al. 2001).

Curcumin is highly metabolized in the intestine and liver, and only small amounts are detectable in other organs. After intraperitoneal administration of curcumin (0.1 g/kg) to mice, 2.25 μg/mL were sampled in the plasma after 15 min. Further, in 1 h after administration, the detected levels of curcumin in the intestine, spleen, liver, and kidneys were 177.04, 26.06, 26.90, and 7.51 mg/g, respectively, and only trace amounts (0.41 mg/g) were observed in the brain (Pan et al. 1999). In another study which assessed curcumin uptake and distribution in animals, Marczylo et al. (2007) found even lower levels of the polyphenol. Oral administration of curcumin (340 mg/kg) in rats gave the following results: plasma (16.1 ng/mL), urine (2.0 ng/mL), intestinal mucosa (1.4 mg/g), liver (3671.8 ng/g), kidney (206.8 ng/g), and heart (807.6 ng/g) (Marczylo et al. 2007). Therefore, species differences, dosage, and sampling of curcumin highly influence its uptake and distribution which are essential for the biological function.

Curcumin undergoes extensive phase I and phase II biotransformation in the intestinal cells and liver (Fig. 3).

Alcohol dehydrogenases present in enterocytes and hepatocytes reduce curcumin to dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin (HHC), and octahydrocurcumin (OHC). These phase I metabolites are found in free and conjugated forms (mostly as glucuronides) (Pan et al. 1999; Ireson et al. 2002). Ireson et al. (2002) investigated curcumin metabolism in human and rat intestinal tissue and in the corresponding hepatic fractions. Once more, species

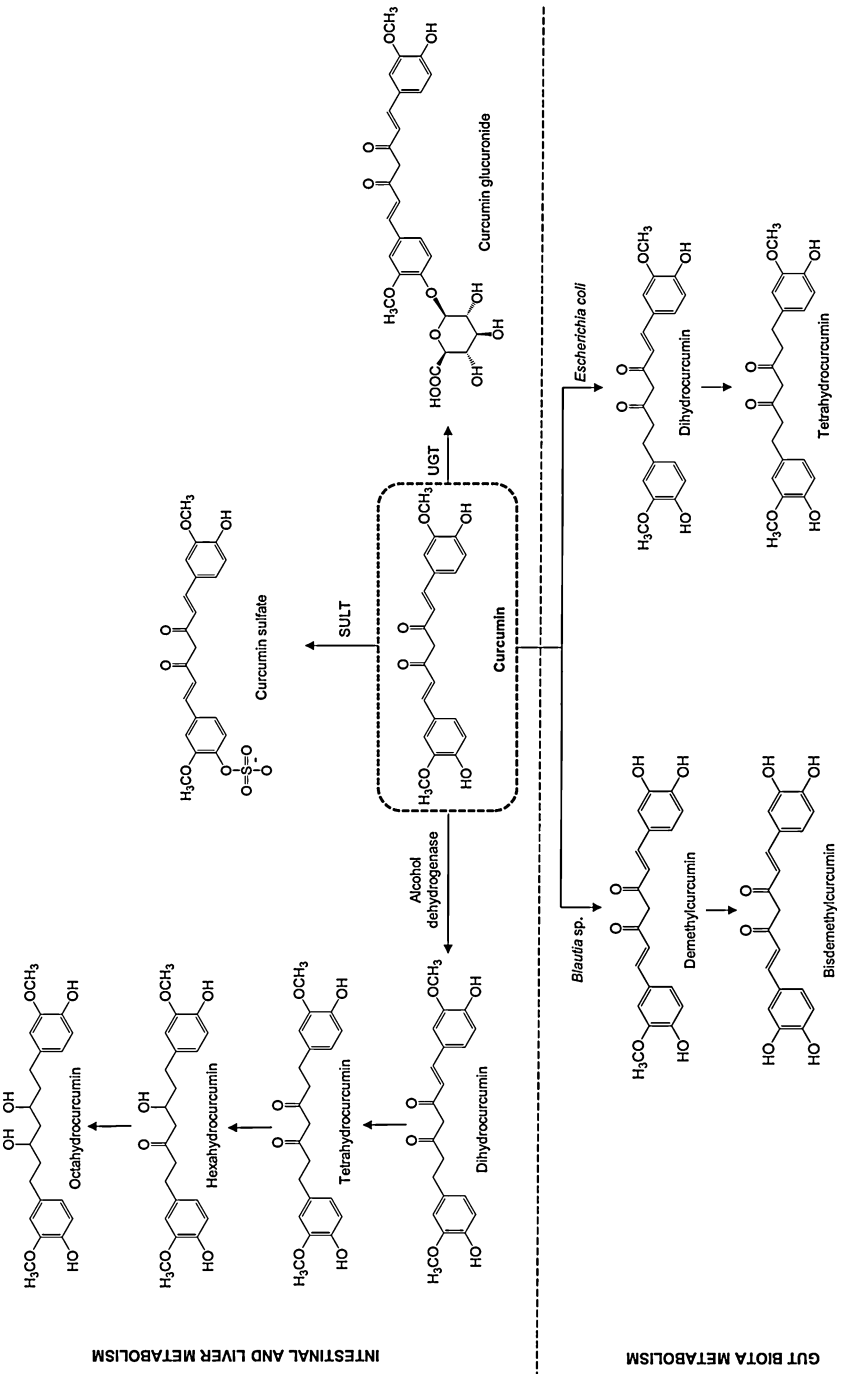


Fig. 3 Major metabolic pathway of curcumin

differences markedly influenced curcumin metabolism. They concluded that the curcumin-reducing ability to HHC in human intestinal and liver tissue cytosol is significantly higher compared to the corresponding rat tissues (by factors of 18 and 5, respectively) (Ireson et al. 2002). More, THC was curcumin major metabolite in the intestinal mucosa, liver, plasma, and urine of rats. In contrast, THC was not detectable in human subcellular fractions (Pan et al. 1999; Marczylo et al. 2007).

Phase II metabolism of curcumin occurs in the intestinal and hepatic cytosol and endoplasmic reticulum microsomes and involves its conjugation with glucuronic acid and sulfate (Ireson et al. 2001, 2002; Garcea et al. 2004; Hoehle et al. 2007). Curcumin is sulfated by sulfotransferases (SULTs), mostly SULT1A1 and SULT1A3 (Ireson et al. 2002). Curcumin sulfation occurs in the cytosol of enterocytes and hepatocytes, and the concentration of sulfated curcumin in the human intestine is threefold higher than in the liver (Ireson et al. 2002).

Glucuronidation of curcumin is catalyzed by uridine 5′-diphospho-glucuronyltransferases (UGTs) in the intestinal and hepatic microsomes (Ireson et al. 2001, 2002). In rats, oral administration of curcumin (500 mg/kg b.w.) led to curcumin glucuronide and curcumin sulfate as major metabolites, alongside HHC, OHC, and HHC glucuronide which were present in small amounts (Ireson et al. 2001). In humans, oral administration of curcumin gives glucuronide and sulfate conjugates as main metabolites, summing almost 100% (Sharma et al. 2004). More, plasma levels of curcumin glucuronides are almost twofold higher than those of sulfate conjugates (Vareed et al. 2008). These results are consistent with the ones from clinical trials conducted by Sharma et al. (2004) and Kunati et al. (2018). Moreover, curcumin and its phase I metabolites can be conjugated by phase II xenobiotic-metabolizing enzymes to monoglucuronides, monosulfates, and mixed glucuronide/sulfates (Garcea et al. 2005).

Excretion of curcumin is mainly done in urine as glucuronide and sulfate conjugates (Asai and Miyazawa 2000; Sharma et al. 2004). After oral administration in humans, curcumin was not detected or was found in minute quantities in feces or urine (Sharma et al. 2001; Schiborr et al. 2014). Thus, curcumin levels sampled on the 8th day from patients consuming 3.6 g of curcumin/day were in the nanomolar range (25–116 nmol/g dried feces) (Sharma et al. 2004).

Curcumin bioactive metabolites are likely to be formed at trace levels in biological samples; therefore their detection and identification represent a real conundrum.

Similarly to other dietary polyphenols, curcumin undergoes an alternative metabolism by intestinal microbiota (Hassaninasab et al. 2011; Lou et al. 2015; Burapan et al. 2017). Studies on curcumin-converting microorganisms were undertaken to unravel the fate of ingested curcumin once it reaches the colon, which presents a unique population of indigenous bacteria.

Escherichia coli is one of the most active curcumin-metabolizing microorganisms isolated from human feces. Thus, curcumin is metabolized by a NADPH-dependent reductase in a two-step reduction pathway (curcumin → DHC → THC) (Fig. 3) (Hassaninasab et al. 2011). *Blautia* sp. MRG-PMF1 is also involved in the gut metabolism of curcumin and produces its demethylation to demethylcurcumin and

bisdemethylcurcumin (Fig. 3) (Burapan et al. 2017). In order to have a representative range of gut bacteria, Lou et al. (2015) used in their study feces sampled from a single male human. After incubation, there were identified novel metabolites derived from curcumin by demethoxylation, reduction, hydroxylation, methylation, and acetylation processes.

To summarize, curcumin is a hydrophobic polyphenol, practically insoluble in water. It presents a reduced intestinal absorption and limited tissue distribution, followed by a rapid metabolism. This translates into a short half-life and explains the low systemic bioavailability of curcumin. Therefore, the bioactivation hypothesis could explain the polypharmacology of curcumin.

In order to overcome the limitations of curcumin's low bioavailability, several pharmacokinetic strategies have been developed. Thus, association of curcumin with bioenhancers such as piperine, quercetin, genistein, eugenol, epigallocatechin gallate, and resveratrol was found to inhibit curcumin metabolism via modulation of phase II enzymes, with a consequent increase in its bioavailability and efficacy. As an illustrating example, piperine, the major alkaloid found in *Piper nigrum*, increased by 20-fold the bioavailability of curcumin, as proven in animal and human studies (Mahran et al. 2017; Shoba et al. 1998).

In addition, synthesis of curcumin analogs and development of modified drug-delivery systems, including liposomal, nanoparticulated, and phospholipid complex formulations of curcumin, has proven increased effectiveness when compared to simple curcumin, as assessed in numerous animal models and clinical studies. Moreover, recent formulations of curcumin have been reported with 100-fold higher bioavailability than curcumin itself, when studied in clinical trials. Thus, modern technologies find their application in the resolution of curcumin's low bioavailability (Khan et al. 2019; Prasad et al. 2014; Marczylo et al. 2007).

27.4 Bioactivities (Animal Aspects)

Numerous animal studies have shown that curcumin possesses various pharmacological effects including antitumor, anti-inflammatory, antioxidant, antidiabetic, hypolipidemic, hepatoprotective, cardioprotective, wound healing, nephroprotective, and neuroprotective effects (Aggarwal et al. 2007; Gupta et al. 2012; Akbik et al. 2014; Kunnumakkara et al. 2017). Scientific literature on assessment of curcumin bioactivity abounds in animal studies; therefore experimental reports cited in the following sections should be considered as illustrative rather than comprehensive.

27.4.1 Anticancer Effects

The chemopreventive, chemosensitizing, and chemotherapeutic properties of curcumin have been investigated in different animal (mostly rodent) models. Curcumin has been found to be effective in many phases of cancer development,

to suppress malignant transformation, beginning, development and invasion of tumor, angiogenesis, and metastasis (Anand et al. 2007).

Chemopreventive efficacy of curcumin for colon cancer has been shown in different rodent models, alongside other cancers located at gastrointestinal level such as esophageal, stomach, liver, and oral cancer. Curcumin also showed chemopreventive effects in animal models of extraintestinal cancers, including the breast, lung, kidney, bladder, blood, and skin (Aggarwal et al. 2007; Hatcher et al. 2008; Epstein et al. 2010).

Heterotransplantation of human cancer cells (xenograft models) into immunodeficient rodents was frequently used for preclinical screen of curcumin as a novel chemotherapeutic agent. Curcumin showed antitumor efficacy against human melanoma cell xenografts when administered intraperitoneally (25 mg/kg b.w.), with an increase in median survival time of 45.7% compared to untreated group (Odot et al. 2004). The authors concluded that the inhibitory effects were mostly due to the antiangiogenic properties of curcumin. More, the combination treatment of curcumin associated with immunization of soluble proteins from supernatants of human melanoma cells was studied. This therapy proved to be more effective, with consequent delay of tumor growth and enhancement of the immune response, that translated into a significantly increase in median survival time of 82.8% (Odot et al. 2004).

When given orally (2% in the diet for 6 weeks) curcumin inhibited cell proliferation and angiogenesis and induced apoptosis in a xenograft nude mice model of prostate cancer (Dorai et al. 2001). Also, curcumin reduced by 50% the volume of xenografts compared to controls in a prostate cancer model, after oral administration of 5 mg/day, 5 days per week for 4 weeks. Analysis of the tumors revealed that curcumin downregulated the expression of murine double minute 2, a major ubiquitin E3 ligase of tumor suppressor p53. More, curcumin enhanced the antitumor effects of gemcitabine and radiation (Li et al. 2007).

Curcumin administered orally (1 g/kg b.w.) in combination with gemcitabine (25 mg/kg b.w.) proved antitumor effects in an orthotopic nude mice model of pancreatic cancer. Curcumin acted as a chemosensitizing agent and potentiated the antitumor effects of gemcitabine by suppressing proliferation (with decrease of tumors volume), angiogenesis, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and NF-κB-regulated gene products [cyclin D1, c-myc, Bcl-2, Bcl-xL, cellular inhibitor of apoptosis protein-1, cyclooxygenase-2 (COX-2), matrix metalloproteinase (MMP), and vascular endothelial growth factor] (Kunnumakkara et al. 2007).

Curcumin acted as chemosensitizer in combination with other chemotherapeutic drugs such as paclitaxel and docetaxel in xenograft models, showing potential in preventing breast cancer metastasis to the lung and inhibiting tumor growth and angiogenesis in ovarian cancer, respectively (Epstein et al. 2010).

Curcumin and its phase I metabolite THC were evaluated for their chemotherapeutic efficacy in a rodent colon cancer model. THC was found to be more active than its parent compound in inhibiting aberrant crypt foci (ACF) development and cell proliferation in colons of mice exposed to 1,2-dimethylhydrazine dihydrochloride (number

of ACF/mouse was 46.6 ± 17.7 vs. 53.3 ± 10.2) (Kim et al. 1998). In contrast, THC was less active than curcumin in a 12-O-tetradecanoylphorbol-13-acetate (TPA)-initiated mouse skin carcinogenesis model, as it inhibited in a less extent than its progenitor the TPA-induced ornithine decarboxylase activity and tumor promotion (Huang et al. 1995).

More, curcumin and another phase I metabolite HHC were proven to possess anticarcinogenic effects in a dimethylhydrazine-induced rat colon cancer model. HHC reduced the number of ACF in rat colon more effectively than curcumin (1086.80 ± 53.47 vs. 1284.20 ± 25.47), activity mediated by downregulation of COX-2 mRNA expression and induction of apoptosis in epithelial cells of focal crypts. More, both curcumin and HHC acted as chemosensitizers in conjunction with 5-fluorouracil. The synergistic effect of HHC in combination with 5-fluorouracil was superior to that of its progenitor with the chemotherapeutic agent (number of ACF was 665.80 ± 16.64 vs. 880.20 ± 13.67) (Srimuangwong et al. 2012).

Curcumin and its phase II metabolite, curcumin sulfate, have been investigated regarding their antitumor effects. Curcumin sulfate was less active than curcumin in inhibiting prostaglandin E_2 activity in an animal model indicating higher efficacy of curcumin in comparison with its bioactive metabolite (Ireson et al. 2001).

27.4.2 Antidiabetic Effects

Curcumin has also been shown to improve the symptoms associated with diabetes. The ability of curcumin in improving glycemic status [blood glucose, glucose tolerance, and glycosylated hemoglobin (HbA1c)], insulin sensitivity, and normalization of lipid profile was assessed in various animal models.

The most used animals in studying the effect of curcumin are rodents (rat and mouse). Various diabetic rodent models were employed to investigate the effect of curcumin on glycemia. In alloxan, streptozotocin (STZ), and STZ-nicotinamide-induced diabetes rat models, oral administration of various dosages of curcumin (from 60 to 300 mg/kg b.w.) for a period of time of 2 weeks up to 8 weeks resulted in a significant reduction in levels of glucose, hemoglobin (Hb), and HbA1C in blood and prevented body weight loss (Zhang et al. 2013).

El-Moselhy et al. (2011) investigated the effect of curcumin on the progression of insulin resistance and type 2 diabetes mellitus (T2DM) induced by a high-fat diet in rats. Oral administration of curcumin (80 mg/kg b.w.) as pretreatment for 60 days and treatment for 15 days showed an antihyperglycemic effect and improved insulin sensitivity. These results were attributed at least in part to curcumin anti-inflammatory properties as evident by attenuating tumor necrosis factor (TNF)-α levels and to its anti-lipolytic effect as evident by lowering plasma-free fatty acids. Curcumin effects were comparable to those of peroxisome proliferator-activated receptor (PPAR)-γ agonist rosiglitazone, a known antidiabetic drug, which indicated that the mechanism of activity of these compounds might be similar (El-Moselhy et al. 2011).

Curcumin reduced inflammation and oxidative stress in the muscle of fructose-fed rats by avoiding the degradation of the inhibitor of kappa B (IkB)-α and decreasing the oxidative stress-sensitive kinases [extracellular signal-regulated kinases (ERK) 1/2 and p38]; consequently, it prevented the activation of the NF-kB pathway and the subsequent production of pro-inflammatory cytokines such as TNF-α and C-reactive protein (Maithilikarpagaselvi et al. 2016).

Curcumin was shown to interfere with glucose metabolism via phosphatidylinositol 3-kinase/protein kinase B/glycogen synthase kinase 3b (PI3K/Akt/GSK-3b) pathway, whose malfunctioning is associated with the development of metabolic disorders (Bustanji et al. 2009; Yu et al. 2012, 2016). Inhibition in a dose-dependent manner of GSK-3b which phosphorylates glycogen synthase by curcumin translated into an increased glycogen synthesis in the liver of fasting mice. Thus, curcumin enhances glucose disposal via increasing the rate of glycogen synthesis in the liver (Bustanji et al. 2009). Similar beneficial effects were observed in another T2DM rodent model. Curcumin treatment (oral doses of 100 or 200 mg/kg b.w./day for 16 weeks) inhibited phosphorylation of Akt and GSK-3b. GSK-3b is a critical downstream element of Akt whose activity can be inhibited by Akt-mediated phosphorylation. As a result, curcumin reduced glucose blood levels and myocardial dysfunction and other parameters such as fibrosis, oxidative stress, inflammation, and apoptosis in animals heart (Yu et al. 2012, 2016).

Murugan and Pari (2007) evaluated the hypoglycemic and antioxidant activities of curcumin and its phase I metabolite THC in a T2DM rat model. Oral dosing of 80 mg/kg b.w. for 45 days led to a significant reduction in blood glucose levels and HbA1c, alongside with a significant increase in plasma insulin levels and activities of erythrocyte antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), reduced glutathione (GSH)]; also, a significant decrease in thiobarbituric acid reactive substances and hydroperoxide formation in the liver and kidney were noticed. THC was found to be more potent than its parent compound curcumin (Murugan and Pari 2007).

27.4.3 Anti-inflammatory Effects

Several studies in various rodent disease models provide strong preclinical evidence for the benefit of curcumin as anti-inflammatory agent.

Curcumin was shown to attenuate colitis in a dinitrobenzene sulfonic acid (DNB)-induced murine model of colitis. Curcumin added to mice diet at a concentration of 0.25%, 5 days prior the DNB instillation, was able to attenuate experimental colitis through a mechanism correlated with the inhibition of the NF-κB activation and reduction in the activity of p38 group of mitogen-activated protein kinases (MAPK), which are involved in the production of pro-inflammatory cytokines and chemokines (Salh et al. 2003).

In a study investigating the protective effect of curcumin (50 mg/kg b.w. in gavage, daily for 10 days) against trinitrobenzene sulfonic acid-induced colitis in

mice, curcumin pretreatment was associated with significant decreases in diarrhea and in disruption of the colonic architecture. Administration of curcumin in similar doses after colitis inducement for 8 more days significantly reduced the degree of both neutrophil infiltration and lipid peroxidation and decreased serine protease activity. Curcumin also reduced the levels of nitric oxide (NO) and suppressed the NF-kB activation in colonic mucosa (Ukil et al. 2003).

Curcumin exerted significant anti-inflammatory effects against colon inflammation in a mouse model of human inflammatory bowel disease. By using a combined transcriptomics and proteomics approach, the mechanism of curcumin activity against inflammation was unraveled and included the decrease of immune response and increase of xenobiotic metabolism, with resolution of inflammation via decreased neutrophil migration and increased barrier remodeling (Cooney et al. 2016).

The effect of curcumin in combination with a subtherapeutic dose of methotrexate was investigated in an induced arthritis rat model. Intraperitoneal administration of methotrexate (1 mg/kg b.w.) along with low and high dose of curcumin (30 mg/kg b.w. and 100 mg/kg b.w.) for 5 weeks exerted a synergistic anti-inflammatory effect, by significantly reducing the volume of paw edema associated with arthritis. More, curcumin minimized the toxicity of methotrexate, by reduction in hepatocellular injury as assessed by lowering the serum levels of glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and bilirubin (Banji et al. 2011).

Jeengar et al. (2016) investigated the anti-arthritic properties of a combination consisting of curcumin and emu (*Dromaius novaehollandiae*) oil. Emu oil increased the permeation of lipophilic curcumin through the skin, with a marked decrease in pro-inflammatory mediators levels such as interleukin (IL)-1β, TNF-α, and IL-6 (Jeengar et al. 2016).

The efficacy of a topically applied curcumin-loaded oleic acid-based polymeric bandage was assessed in a wound rat model. The treatment targeted the NF-κB pathway; curcumin downregulated the expression of various kinases involved in the NF-κB pathway and upregulated the expression of the IkB-α, which led to less activation of the NF-κB gene and reduced inflammation (Mohanty et al. 2012).

The anti-inflammatory activity of curcumin and its phase I metabolite THC was investigated by carrageenan-induced rat paw edema assay and cotton pellet granuloma formation test. At low doses, THC was more effective than its progenitor in suppressing inflammation; however, at higher doses, this effect was only partially reversed. THC completely lacked activity in the cotton pellet granuloma formation test (Mukhopadhyay et al. 1982).

27.4.4 Wound Healing Effects

There is evidence supporting the wound healing efficacy of curcumin, as assessed by animal studies. It seems that the wound healing capacity of curcumin is attributed mainly to its anti-inflammatory, anti-infectious, and antioxidant effects.

Curcumin also enhances wound healing by stimulation of tissue remodeling, granulation tissue formation, and collagen deposition. More, even topical use of curcumin promotes epithelial regeneration and increases fibroblast proliferation and vascular density (Akbik et al. 2014).

The in vivo effects of curcumin on wound healing were examined in rats and guinea pigs by Sidhu et al. (1998). Thus, curcumin was administered in doses of 40 mg/kg b.w. for 6 up to 11 days, either orally to guinea pigs or injected intraperitoneally to rats. Punch wounds in curcumin-treated animals closed faster compared to untreated animals. More, curcumin enhanced epithelial regeneration and collagen deposition and increased vascular density of dermis and migration of myofibroblasts, fibroblasts, and macrophages in the wound bed. Moreover, immunohistochemical studies revealed that curcumin increased the levels of transforming growth factor (TGF)-β1, thus demonstrating that the polyphenolic compound acts by modulating the TGF-β1 activity, known as a wound healing promoter (Sidhu et al. 1998).

Mani et al. (2002) investigated the effect of curcumin-dexamethasone combination treatment by topical application in a punch wound rat model. Curcumin significantly enhanced wound healing independently of dexamethasone as compared to controls. Its mechanism of activity was based on enhancement of TGF-β1 expression and increase of inducible nitric oxide synthase (*i*NOS) levels (Mani et al. 2002). It has been shown that increased NO production promotes wound healing by enhancing inflammation (Akbik et al. 2014), fact supported by another animal study conducted by Jagetia and Rajanikant (2012) which also concluded that an increase in NO was partially responsible for the improvement in wound healing by curcumin treatment.

Literature reports suggest that topical application of curcumin has been shown to cause a significant reduction in the expression of antioxidant enzymes in the wound tissue, as curcumin reduces oxidation through nonenzymatic mechanisms (Akbik et al. 2014).

Formulation of curcumin in a collagen matrix was investigated for its topical wound healing in a rat model. Curcumin slightly raised CAT activity but determined a significant decrease of SOD level in treated group compared to control. This decrease in SOD was attributed to antioxidant activities of curcumin, which nonenzymatically reduces superoxide radicals and consequently decreases oxidative stress and antioxidant enzymes level (Gopinath et al. 2004). Similarly, Mohanty et al. (2012) also reported downregulation of SOD, CAT, and GPx expression in a rat model, following the treatment of excised wounds with a curcumin polymeric formulation. This was also due to the reactive oxygen species (ROS) scavenging ability of curcumin, which reduces activation of antioxidant enzymes.

27.4.5 Neuroprotective Effects

Mounting evidence indicates curcumin efficacy in various animal models of neurological disorders. Several studies in rodents have proved the neuroprotective

effects of curcumin in neurodegenerative disorders, especially Alzheimer's and Parkinson's diseases.

Agarwal et al. (2011) investigated the effect of orally administered curcumin (acute administration of 50, 100, and 200 mg/kg b.w. doses) in a model of pentylenetetrazole-induced kindling in mice. Curcumin suppressed dose-dependently the progression of kindling in mice, by reducing the levels of malondialdehyde (MDA) and glutathione in kindled animals. Therefore, curcumin acted as an antiepileptic agent, controlling both development of seizures and oxidative stress during epilepsy (Agarwal et al. 2011). Similar effects were demonstrated in other two models of seizures induced in rats by pentylenetetrazole and kainic acid. Pretreatment with curcumin led to less severe seizures and less cognitive impairment in rats compared to control group (Gupta et al. 2012).

The neuroprotective efficacy of curcumin given intraperitoneally post-ischemia (300 mg/kg b.w., after 4 h of clot implant) was assessed in a rat thromboembolic stroke model. Curcumin treatment selectively inhibited the generation of cytotoxicity factors such ROS, NO, and peroxynitrite in neuronal-rich cell population. Also, it significantly reduced brain infarct volume and edema volume. Sensory motor function, namely, locomotor activity and motor coordination, were improved by curcumin, as assessed by neurobehavioral testing (Dohare et al. 2008).

Garcia-Alloza et al. (2007) demonstrated that curcumin treatment for 7 days (tail vein injections in doses of 7.5 mg/day) reduced plaque formation and β-amyloid accumulation in a mouse model of Alzheimer's disease. Systemic treatment with curcumin cleared and also reduced existing plaques, suggesting a potent disaggregation effect. Curcumin also led to a limited but significant reversal of structural changes in dystrophic dendrites, including abnormal curvature and dystrophy size. The authors concluded that curcumin crosses the blood-brain barrier and reverses existing amyloid pathology and associated neurotoxicity (Garcia-Alloza et al. 2007). More, dietary curcumin also attenuated oxidative injury, microgliosis, synaptophysin loss, spatial memory deficits, postsynaptic loss, and β-amyloid deposits produced by intracerebral ventricular infusion of β-amyloid in rats (Frautschy et al. 2001). These results confirmed the capacity of curcumin in reducing amyloid levels and plaque burden in experimental animal models, thus proving the putative clinical efficacy against Alzheimer's disease (Gupta et al. 2012; Amalraj et al. 2016; Sarker and Franks 2018).

Neuroprotective effects of curcumin were investigated in a 6-hydroxydopamine-treated rat model of Parkinson's disease. Rats pretreated with curcumin (50 mg/kg b.w./day by gavage, for 4 days) showed clear protection of dopamine in the striatum due to attenuation of dopaminergic neurons loss and restoration of dopamine striatal concentration. Curcumin neuroprotective effects were related to its antioxidant activity and ability to penetrate into the brain (Zbarsky et al. 2005). Several studies evaluated the protective effect of curcumin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease. Curcumin was shown to substantially improve behavioral deficits and enhance neuron survival in the substantia nigra; the polyphenol significantly reversed the MPTP-induced

depletion of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid via inhibition of monoamine oxidase (MAO-B) activity (Rajeswari and Sabesan 2008).

Curcumin has shown protective effects in other animal models of neurological disorders such as diabetic encephalopathy, encephalomyelitis, intracerebral hemorrhage, spinal cord injury, cerebral malaria, convulsions, and brain ischemia (Amalraj et al. 2016).

27.4.6 Cardioprotective Effects

Animal studies have provided evidence suggesting protective effects of curcumin against diverse cardiovascular pathological states leading toward heart failure.

Liu et al. (2017) investigated the protective effect of curcumin on heart function in an ischemia reperfusion injury model. Curcumin pretreatment (10, 20, or 30 mg/kg b.w./day, for 20 days) before coronary artery ligature (60 min), followed by reperfusion, showed significant reductions in oxidative stress, infarct size, and inhibition of myocardium apoptosis by decreasing the expression of apoptotic genes in rats (Liu et al. 2017). Similarly, curcumin pretreatment (200 mg/kg b.w. per day orally for 10 days) proved cardioprotective activity in another ischemia reperfusion injury model by activating signaling pathways which reduce mitochondrial damage. Curcumin improved postischemic cardiac function, decreased myocardial infarct size, and decreased myocardial apoptotic index and several biochemical parameters, including the upregulation of the antiapoptotic proteins and the downregulation of the pro-apoptotic proteins. More, curcumin acted by preserving mitochondrial redox potential, with significant elevation of mitochondrial SOD activity, and by decreasing the formation of mitochondrial hydrogen peroxide and MDA (Yang et al. 2013).

In the study carried out by Abo-Salem et al. (2014), curcumin showed protective effects in diabetes-induced cardiac injury in rats. Orally administered curcumin (200 mg/kg b.w./day, for 6 weeks) markedly decreased serum high-density lipoprotein cholesterol and increased cardiac antioxidant enzymes (CAT, SOD, and GST). The authors attributed these effects to curcumin to its hypolipidemic, free radical scavenging, and anti-inflammatory activities (Abo-Salem et al. 2014).

Xiao et al. (2016) investigated the role of curcumin and its mechanism of action on interstitial fibrosis after myocardial infarction (MI) in mice. Curcumin pretreatment (100 mg/kg b.w./day orally for 7 days) significantly attenuated collagen deposition and inhibited the cardiac fibroblasts proliferation and migration. In addition, curcumin inhibited MMP-induced extracellular matrix degradation and disallowed the downregulation of sirtuin 1 (SIRT1) after MI. The study provided evidence for the protective role of curcumin against myocardial fibrosis, which was partially mediated by SIRT1 activation (Xiao et al. 2016).

Curcumin exerted protective effects in an atherosclerosis mice model. Administration of curcumin (oral dose of 0.3 mg/per day/per mouse) combined with a high-fat diet decreased the lesion formation by approx. 20% (Olszanecki et al. 2005).

Curcumin has also been shown to improve cardiac function via upregulating the expression of sarcoplasmic reticulum Ca^{2+}-ATPase in a rabbit model (Zhang et al. 2013).

Nakmareong et al. (2011) investigated whether curcumin and its phase I metabolite, THC, could prevent vascular dysfunction and inhibit development of hypertension in N^{ω}-nitro-L-arginine methyl ester-treated rats. Dietary curcumin and THC (50 and 100 mg/kg/b.w./day for 3 weeks) significantly suppressed blood pressure elevation, decreased vascular resistance, and restored vascular responsiveness, without affecting these parameters in normotensive rats. These effects were mostly due to induction of endothelial nitric oxide synthase (*e*NOS) protein expression in the aortic tissue and increase of GSH blood levels, corroborated with a decrease in oxidative stress. The antihypertensive and some antioxidant effects of THC were more potent than those of curcumin (Nakmareong et al. 2011).

27.4.7 Anti-obesity Effects

Animal studies showed improved lipid profiles following curcumin treatment (Gupta et al. 2012; Rivera-Mancía et al. 2018).

Curcumin potential to prevent obesity was investigated in a mouse model. Supplementation of the high-fat diet with 500 mg/kg b.w. curcumin for 12 weeks reduced body weight gain, adiposity, and suppressed angiogenesis in adipose tissue. Curcumin reduced expression of vascular endothelial growth factor (VEGF) and VEGF receptor 2, important factors in angiogenesis progression. Also, it reduced peroxisome proliferator-activated receptor c and CCAAT/enhancer-binding protein-α, key transcription factors in adipogenesis and lipogenesis (Ejaz et al. 2009).

In animals fed a high-fat diet, dietary curcumin has been shown to decrease serum cholesterol levels, by modulation of enzymes involved in cholesterol uptake and transport in the intestine (acyl-CoA:cholesterol acyltransferase) and transformation of cholesterol into bile acid (cholesterol 7-hydroxylase) (Kim and Clifton 2018).

Curcumin treatment (80 mg/kg b.w./day by oral gavage) for 12 weeks ameliorated high-fat diet (HFD)-induced body weight gain and fat accumulation in liver and adipose tissues and improved serum lipid levels and insulin sensitivity in HFD-induced obese mice. Curcumin regulated the expression of sterol regulatory element-binding proteins (SREBPs) in the liver and adipose tissue, genes which are involved in biosynthesis of cholesterol, fatty acids, and triglycerides (Ding et al. 2016).

Antioxidant effects of curcumin significantly contribute to its anti-obesity activity, as assessed in rabbits models. Administration of curcumin was found to reduce the contents of lipid and thiobarbituric acid reactive substances and to enhance GPx and CAT activities in the liver of purely cholesterol-fed rabbits (Gupta et al. 2012).

Curcumin improved the lipid profile in animals with hyperlipidemia, metabolic syndrome and diabetes, with a decrease in plasma triglycerides and non-high-density

lipoprotein-cholesterol, and an increase in high-density lipoprotein (HDL)-cholesterol levels (Rivera-Mancía et al. 2018).

Curcumin was also shown to inhibit leptin activity in several animal models. It is known that leptin inhibits the appetite and contributes to insulin sensitivity. However, in the context of coexistent obesity/hyperleptinemia/diabetes mellitus, leptin resistance may occur, leading to overfeeding, fat accumulation, and alteration of lipid and glucose metabolism (Rivera-Mancía et al. 2018).

27.4.8 Hepatoprotective Effects

Curcumin exerts protective effects in liver diseases, as assessed in various animal studies. Liver damage can be induced by numerous agents, with progression of liver injury, nonalcoholic steatohepatitis, nonalcoholic liver disease, liver fibrosis, and cirrhosis. The beneficial effects of curcumin in liver diseases can be due to its anti-inflammatory, antioxidant, and antifibrogenic properties. Curcumin acts as a free radical scavenger of ROS mostly via its phenolic, diketone, central methylenic, and methoxy groups (Khan et al. 2019; Farzaei et al. 2018).

Oral supplementation with curcumin 18 mg/day for 30 days alleviated non-alcoholic steatohepatitis (NASH) in rabbits. Curcumin decreased aminotransferases activity and increased the mitochondrial antioxidants. More, curcumin also reduced mitochondrial ROS, improved mitochondrial function, and suppressed the activation of NF-kB pathways, including inhibition of TNF-α production and release. Contrarily, similar doses of vitamin E were unable to reduce NASH. Thus, curcumin exerted hepatoprotective effects through a mechanism involving antioxidant, anti-inflammatory, and mitochondrial-protective activities (Ramirez-Tortosa et al. 2009). In agreement with this study, several other animal models with experimental-induced steatohepatitis showed that the hepatoprotective effects of curcumin are related with downregulation of NF-κB transcription factor, an improvement of hepatic fibrosis also in alcoholic liver injuries, an increase of animal survival rates, and a reduction of oxidative damage (Khan et al. 2019; Pulido-Moran et al. 2016).

The efficacy of curcumin in a lipopolysaccharide (LPS)/D-galactosamine-induced acute liver injury (ALI) in rats was studied by Xie et al. (2017). Curcumin pretreatment (orally given by gavage in doses of 30, 60, and 120 mg/kg b.w. for 3 days prior ALI inducement) attenuated hepatic pathological damage, reduced serum levels of alanine transaminase (ALT) and aspartate transaminase (AST), and reduced MDA content in experimental ALI rats. These effects relied on improvement of inflammation and liver pathology. Higher dosages of curcumin inhibited NF-kB activation and reduced serum and liver levels of inflammatory cytokine TNF-α and upregulated the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and Nrf2-dependent antioxidant defense genes causing cellular resistance to oxidative stress (Xie et al. 2017).

In CCl_4-induced hepatic damage, curcumin (200 mg/kg b.w./day) administered intragastrically to rats for 3 days decreased aminotransferases (ALT and AST) level, suppressed lipid peroxidation, reduced MDA levels, and improved the level of serum reduced and oxidized glutathione (GSH and GSSG) ratio. Curcumin also showed protective effects against decreased SOD and GPx activities, with a recovery of redox balance and an increase of GSH level. Based on these findings, the authors suggested that hepatoprotective effects of curcumin are mostly due to its antioxidant activity; in addition, curcumin might play a role in restoring the liver redox capacity, as represented by GSH/GSSG cycling capacity (Lee et al. 2017).

27.4.9 Nephroprotective Effects

The nephroprotective potential of curcumin, based mostly on its anti-inflammatory, antiproliferative, and antioxidant activities, was reported by several studies which employed animal models. Curcumin exerts positive effects in terms of cardiovascular and nephron protection, thus alleviating inflammation and oxidative stress, which contribute to the increased morbidity and cardiovascular mortality associated with chronic kidney disease (CKD) (de Almeida Alvarenga et al. 2018; Rauf et al. 2018).

Curcumin has shown nephroprotection in a five-sixths nephrectomy (5/6NX)-induced renal damage rats model. Curcumin pretreatment (60 mg/kg/day by oral administration 7 days before 5/6 NX) decreased blood urea nitrogen, plasma creatinine, and renal vascular resistance and increased glomerular filtration rate and renal blood flow. Curcumin-pretreated group showed a higher concentration of endogenous antioxidants such as lipoic acid, ubiquinone, plasmalogen, glutathione, melatonin in the rats kidney, and mitochondria. Also, curcumin pretreatment decreased alterations in mitochondrial dynamics, bioenergetics, and oxidative stress in nephrectomized rats, suggesting that curcumin might contribute to preservation of renal function (Aparicio-Trejo et al. 2017).

Ali et al. (2017) reported nephroprotective efficacy of curcumin in an adenine-induced CKD rats model. Thus, daily gavage of curcumin at doses of 37.5, 75, and 150 mg/kg b.w. for 35 days were associated with alleviation of elevations in blood pressure, urinary albumin/creatinine ratio, plasma urea, and creatinine. Inflammatory markers such as IL-1β, IL-6, and TNF-α, alongside morphological damage and histopathological markers of inflammation, fibrosis, and apoptosis, were significantly reduced by curcumin at higher dosage. These results were accompanied by an increased expression of Nrf2, as well as the activity of its direct target glutathione reductase (GR) and of an indirect target, the GSH level (Ali et al. 2017).

Curcumin reversed glomerular hemodynamic alterations and structural damage induced by oxidative stress in 5/6NX rats. Administration by oral gavage of 60 and

120 mg/kg b.w. curcumin doses, 7 days before and 60 days after 5/6NX, were able to attenuate proteinuria and to reverse renal structure such as interstitial fibrosis, fibrotic glomeruli, tubular atrophy, and mesangial expansion. In addition, curcumin was able to decrease hemodynamic changes such as glomerular hypertension and hyperfiltration. More, curcumin enhanced translocation of Nrf2, with attenuation of oxidative stress and preservation of the activity of several antioxidant enzymes such as CAT, GPx, GR, and GST (Tapia et al. 2013).

In several animal studies, curcumin and theracurmin, a novel formulation of curcumin with enhanced bioavailability, were reported to have protective effects against cardiovascular disease, commonly associated with chronic kidney disease (CKD). Both attenuated oxidative stress-related events such as cardiac remodeling, mitochondrial dysfunction, and cell death (de Almeida Alvarenga et al. 2018).

27.4.10 Prevention of Respiratory Disorders

The effects of curcumin in prevention of respiratory disease are based on modulation of inflammatory pathways and prevention of oxidative damage, by decreasing inflammatory cells accumulation and cytokines overproduction and increasing ROS scavengers activity (Pulido-Moran et al. 2016).

Curcumin was evaluated for its ability to suppress pulmonary injuries induced by bleomycin and amiodarone in models of induced pulmonary fibrosis in rats. Oral administration of curcumin as pretreatment and treatment during the period of fibrosis inducement played a protective role against the development of pulmonary fibrosis by blocking the release of TNF-α and cytotoxic free radicals. Thus, curcumin acted as an antioxidant, anti-inflammatory, and antifibrotic agent, by interfering with the influx of inflammatory cells, by suppressing the activation of alveolar macrophages and subsequent release of toxic mediators, and by preventing excess collagen accumulation in lungs (Punithavathi et al. 2000, 2003).

Similar protective effects for curcumin were reported by Venkatesan (2000) and Venkatesan and Chandrakasan (1995). The authors showed the beneficial effects of curcumin against cyclophosphamide and paraquat-induced pulmonary fibrosis in rats. Oral administration of curcumin (300 mg/kg b.w. for 10 days) reduced the level of leukocytes and inflammatory mediators and increased lung antioxidant defenses. More, curcumin reduced the toxicity and mortality associated with cyclophosphamide and paraquat administration in rats (Venkatesan 2000; Venkatesan and Chandrakasan 1995).

27.5 Benefits (Human Studies)

Numerous clinical studies evaluated the health benefits of curcumin administered as pure compound, mixture of curcuminoids, or highly bioavailable formulations. Most of these studies highlighted a promising therapeutic potential in oxidative stress and inflammation-related disorders.

27.5.1 Curcumin in Malignant Diseases

27.5.1.1 Curcumin in Colorectal Cancer

A non-randomized, open-label clinical trial investigated the prevention potential of oral curcumin in colorectal neoplasia. The trial (phase IIA clinical trial) enrolled 44 smokers with an increased risk of colorectal adenomas (8 or more aberrant crypt focci detected by colonoscopy). Micronized curcumin (4 g daily for 30 days) reduced by 40% the number of aberrant crypt focci. Lower doses (2 g daily for 30 days) were not effective (Carroll et al. 2011). Two human studies investigated the effects of oral curcumin administered as Curcumin C3 Complex (500 mg of curcuminoids (450 mg of curcumin, 40 mg of demetoxycurcumin (DMC), 10 mg of bisdemetoxycurcumin (BDMC))/capsule) in colorectal cancer patients. In a phase I dose-escalation study, 15 patients with advanced colorectal cancer refractory to standard chemotherapies received daily doses of 0.45–3.6 g of curcumin (1, 2, 4, or 8 capsules) for up to 4 months. The daily dose of 3.6 g curcumin induced significant reductions in inducible prostaglandin E2 production in blood samples (62% and 57% on days 1 and 29 of treatment, respectively) suggesting potential benefits in the prevention or treatment of cancers with other location than the gastrointestinal tract (Sharma et al. 2004). On the other hand, Garcea et al. (2005) found that only 7-day administration of the same daily dose of curcumin (3.6 g administered as Curcumin C3 Complex) reduced the level of the oxidative DNA adduct 3-(2-deoxy-β-di-erythro-pentafuranosyl)-pyr[1,2-α]-purin-10(3*H*)one (M_1G), biomarker of colorectal carcinogenesis, in malignant colorectal tissue in 12 colorectal cancer patients. A curcumin preparation (18 mg of curcumin and 2 mg of DMC suspended in 200 mg turmeric essential oils) was investigated in 15 patients with advanced colorectal cancer refractory to standard chemotherapies (dose-escalation pilot study). The preparation was administered in doses of 0.44–2.2 g containing 36–180 mg of curcumin for up to 4 months. Surprisingly, after 29-day administration of a daily dose of 0.44 g (equivalent to 36 mg curcumin), a decrease by 59% in lymphocytic glutathione S-transferase activity (marker of resistance to chemotherapy) was observed, whereas no downregulation in enzyme activity was detected at higher doses (Sharma et al. 2001). Another study investigated the impact of a combination treatment (480 mg of curcumin and 20 mg of quercetin, oral administration, three times daily) in five familial adenomatous polyposis patients who had previously undergone colectomy. After 6-month therapy, both number and size of polyps were significantly reduced (by 60.4% and 50.9%, respectively) (Cruz-Correa et al. 2006). Several years later, Cruz-Correa et al. (2018) reported the lack of efficacy of pure curcumin (1.5 g twice per day, orally, 12 weeks) in 44 patients with familial adenomatous polyposis (no difference in the mean number or size of lower intestinal tract adenomas between curcumin and placebo groups). However, curcumin potential to prevent and/or treat colorectal cancer is supported by its accumulation and persistence in colorectal mucosa. Administration of Curcumin C3 Complex to colorectal cancer patients (2.35 g/day for 14 days before biopsy or tumor resection) resulted in prolonged colonic tissue levels of curcuminoids (mean mucosal level of curcumin: 48.4 μg/g with persistence of up to 40 h after administration) (Irving et al. 2013). The positive effects of oral

curcumin in colorectal tumor tissue have been clinically proved. A double-blind randomized controlled study enrolling 126 colorectal cancer patients reported increase in the expression of pro-apoptotic and tumor suppressor proteins (Bax and p53, respectively) in tumor tissue after 10–30-day administration of 360 mg of curcumin thrice daily. These effects were accompanied by an increase in apoptotic tumor cells and decrease in serum TNF-α (Mantzorou et al. 2018; Salehi et al. 2019).

27.5.1.2 Curcumin in Pancreatic Cancer

A non-randomized, open-label, phase II trial designed by Dhillon et al. (2008) assessed the efficacy of curcumin in advanced pancreatic cancer. Curcumin was given in a mixture with DMC and BDMC (900, 80, and 20 mg, respectively). Twenty-five patients took oral curcuminoid mixture (8 g daily) until disease progression, with restaging every 2 months. The 2-month therapy downregulated the expression of NF-kB, COX-2, and phosphorylated signal transducer and activator of transcription 3 (pSTAT3) in peripheral blood mononuclear cells; NF-kB, COX-2, and pSTAT3 play key roles in tumorigenesis. It is worthy to mention that one patient had stable disease for more than 18 months, whereas another one showed a marked but brief tumor regression (73%) with significant increased levels of IL-6, IL-8, and IL-10 and IL-1 receptor antagonists (4–35-fold). This increase in cytokine levels was attributed to their release from tumor cells due to cell shrinkage. Other trials investigated the activity of the antimetabolite gemcitabine in combination with oral curcumin in pancreatic cancer. An open-labeled phase II study assessed the effectiveness of gemcitabine (1 g/m^2 intravenously weekly for 3 of 4 weeks) in combination with curcumin in 17 patients with advanced pancreatic cancer; curcumin was administered as mixture of curcuminoids (500 mg of curcuminoids, 450 mg of curcumin, 40 mg of DMC, 10 mg of BDMC) at a dose of 8 g/daily. The levels of CA 19–9 tumor marker were measured every 8 weeks; important reductions were detected in three patients (from 90,830, 714, and 214 to 63,130, 96, and 42, respectively) (Epelbaum et al. 2010). Clinical benefit response (CBR), based on changes in pain, Karnofsky performance status and weight (Bernhard et al. 2014), was achieved in four of the ten symptomatic patients (Epelbaum et al. 2010). The phase I/II study designed by Kanai et al. (2011) evaluated the effects of co-administration of curcumin in 21 gemcitabine-resistant pancreatic cancer patients receiving gemcitabine/S-1 combination therapy (19 patients) or gemcitabine (two patients). S-1 is an oral anticancer drug consisting of tegafur, gimeracil, and oteracil (molar ratio 1:0.4:1) (Kobayakawa and Kojima 2011). Curcumin-based complementary therapy (8 g of curcuminoid mixture/day with 900 mg of curcumin, 80 mg of DMC, and 20 mg of BDMC/g of mixture) proved to be safe and feasible. The median survival time was 161 days, and 1-year survival rate was 19%, whereas the median survival time in non-responders to gemcitabine was only 10 weeks (Kanai et al. 2011). Beneficial effects were also found for the combination of Theracurmin, a highly bioavailable curcumin formulation with standard gemcitabine-based chemotherapy in patients with pancreatic (14) and biliary tract (2) cancers who did not respond to gemcitabine. Administration of Theracurmin

(equivalent to 200 and 400 mg of curcumin daily) improved the fatigue- and functioning-associated quality-of-life scores. The median survival time was 4.4 months in patients with pancreatic cancer with three patients surviving for more than 3 months (Kanai 2014).

27.5.1.3 Curcumin in Prostate Cancer

Hejazi et al. (2016) assessed the efficacy of curcumin supplementation in 40 prostate cancer patients during radiotherapy in a double-blinded, randomized, placebo-controlled study. Twenty patients treated with radiotherapy for prostate cancer received curcumin as Biocurcumin (BCM95) (347 mg of curcumin, 84 mg of DMC, 9 mg of BDMC, and 38 mg of turmeric essential oil/capsule) in a dose of 3 g daily 1 week before initiation until completion of radiotherapy (daily fractions of 2 Gy for 5 days/week to achieve a dose of 74 Gy). Curcumin supplementation enhanced plasma total antioxidant capacity (12.8 vs. 10.6 U/mL in placebo group) and reduced superoxide dismutase activity (189.4 vs. 215.2 U/L in placebo group), but it had no effect on prostate-specific antigen (PSA) levels. Both oxidative stress-related markers and PAS levels were determined 3 months after radiotherapy completion. Curcumin-based combination therapy proved to be efficient in prostate cancer patients. A non-randomized, open-label, pilot phase II study was performed to evaluate the efficacy of docetaxel/prednisone in combination with curcumin in 30 patients with chemotherapy-naive metastatic castration-resistant prostate cancer. Within the first three treatment cycles, curcumin (6 g/day for seven consecutive days in each cycle starting 4 days before docetaxel administration till the second day following administration) caused significant reductions in PSA levels (more than 80% in two-thirds of patients) (Mahammedi et al. 2016). Ide et al. (2010) carried out a randomized, double-blind trial enrolling 85 patients without prostatic intraepithelial neoplasia or cancer but having elevated PSA levels. Six-month administration of a combination of curcumin (100 mg/day) and soy isoflavones (40 mg/day) led to a significant decrease in PSA level (from 18.8 to 10.2 ng/mL) in patients having initial PSA $\geq$ 10 ng/mL. Curcumin in combination with soy isoflavones does appear to be beneficial in patients with elevated PSA levels.

27.5.1.4 Curcumin in Breast Cancer

The open-label, phase I trial carried out by Bayet-Robert et al. (2010) on 14 metastatic breast cancer patients showed promising results for curcumin in combination with a standard dose of docetaxel. Curcumin, formulated as 500 mg capsules (450 mg curcumin/capsule), was orally given for seven consecutive days in each cycle starting 4 days before docetaxel administration till the second day afterward, in escalating doses from 0.5 till 8 g/day. In eight patients receiving the combination therapy, marked reductions in levels of carcinoembryonic antigen (CEA) tumor marker and vascular endothelial growth factor (VEGF) were detected after the third cycle of treatment. The reduction of the latter (by 30% at the third cycle and 21% at the sixth cycle) suggests an antiangiogenic effect of the combination. In a randomized, double-blind, placebo-controlled clinical trial, oral curcumin was found to attenuate radiation dermatitis in patients diagnosed with noninflammatory

breast cancer or carcinoma in situ. Thirty patients receiving radiotherapy without concurrent chemotherapy took 2 g of Curcumin C3 Complex (~390 mg of curcumin, 75 mg of DMC, 12.5 mg of BDMC in 500 mg capsule) thrice a day during radiotherapy. Curcumin reduced the severity of radiation dermatitis and presence of moist desquamation but not the redness (Ryan et al. 2013).

27.5.1.5 Curcumin in Head and Neck Squamous Cell Carcinoma

In patients with head and neck cancer, chewing two curcumin caplets (1 g curcumin) for 5 min reduced IkB kinase β (IKKβ) kinase activity in the salivary cells; the enzyme is involved in NF-kB activation and consequently in tumor growth. Curcumin also downregulated the expression of several pro-inflammatory salivary cytokines such as IL-10, IL-12p70, IL-2, γ interferon (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF-α (Kim et al. 2011).

27.5.1.6 Curcumin in Multiple Myeloma

A phase I/II study investigated the effects of 12-week administration of curcumin alone (2, 4, 6, 8, or 12 g/day in two doses) or in combination with bioperine (10 mg in two doses) in 29 multiple myeloma patients. Curcumin induced significant reductions in the activation of NF-kB and STAT3 but also in the expression of COX-2 in patients peripheral blood mononuclear cells (Salehi et al. 2019). Monoclonal gammopathy of undetermined significance (MGUS) is a plasma cell dyscrasia accompanied by paraproteinemia which can progress to multiple myeloma. In a single-blind, crossover pilot study, enrolling 26 patients with MGUS, curcuminoid mixture (900 mg of curcumin, 80 mg of DMC, and 20 mg of BDMC/tablet, 4 g daily, 12 months) reduced the paraprotein level (12–30% reduction) and urinary N-telopeptide of type I collagen (> 25% reduction), marker of osteolysis (Golombick et al. 2009). Golombick et al. (2012) designed a more complex study, a randomized, double-blind, placebo-controlled, crossover study (6 months) followed by an open-label extension study (3 months) to assess the potential of curcumin to slow down the progression of MGUS and smoldering multiple myeloma (SMM) to multiple myeloma; the study enrolled 36 patients (19 MGUS and 17 SMM). Curcumin was given as curcuminoid mixture: 4 g (3.6 g of curcumin, 0.32 g of DMC, 0.08 g of BDMC) and 8 g daily in the crossover and open-label extension studies, respectively. In both MGUS and SMM patients, 4 and 8 g therapy caused significant reductions in several markers for disease diagnosis and monitoring such as the free light chain ratio (rFLC) (35 and 36% reduction, respectively), difference between involved and uninvolved light chains (dFLC) (9 and 11% reduction, respectively), and involved free light chain (iFLC) (8 and 10% reduction, respectively). Curcumin also decreased the urinary deoxypyridinoline (u-DPYD), a marker of bone resorption (9–14% reduction), random urinary protein, and total serum protein (Golombick et al. 2012). Curcumin appears to slow down the progression of MGUS and SMM to multiple myeloma.

27.5.2 Curcumin in Central Nervous System Disorders

27.5.2.1 Curcumin in Alzheimer's Disease

Recent human trials showed that long-term administration of bioavailable curcumin leads to significant improvements in memory and attention. In a 18-month double-blind, placebo-controlled trial, 21 middle-aged and older non-demented adults received 90 mg of bioavailable curcumin (Theracurmin®) twice daily. Bioavailable curcumin improved memory and attention as revealed by Buschke Selective Reminding Test (SRT), Brief Visual Memory Test-Revised (BVMT-R), and Trail Making Test Part A. The same study investigated amyloid and tau proteins accumulation in brain regions by 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile positron emission tomography (FDDNP-PET) and found reduced deposits in subjects receiving bioavailable curcumin. The benefits of bioavailable curcumin, namely, improvements in memory and attention, seemed to be associated with the decrease in amyloid plaques and tau tangles deposits in the brain (Small et al. 2008). Benefits on cognitive function and mood were also proven for a bioavailable solid lipid curcumin formulation (Longvida®) in a daily dose of 400 mg (corresponding to 80 mg of curcumin). In a randomized, double-blind, placebo-controlled, phase III/IV trial including healthy aged adults, curcumin formulation improved sustained attention and working memory tasks (1 and 3 h after a single dose), mood and working memory (after 4 weeks), and alertness and contentedness (1 and 3 h after single dose following chronic treatment) (Cox et al. 2015; Salehi et al. 2019). Hishikawa et al. (2012) reported three cases of 79-, 83-, and 84-year-old patients with progressive dementia or short-term memory loss who took 764 mg of turmeric powder (corresponding to 100 mg of curcumin) daily for 12 weeks with significant decrease in the acuity of irritability, agitation, anxiety, and apathy. In a randomized, double-blind, placebo-controlled trial enrolling 34 Alzheimer's disease patients, once daily administration of curcumin (4 or 1 g) in combination with a standardized *Ginkgo biloba* leaf extract (120 mg) for 6 months caused an increase in amyloid-β 40 (Aβ40) which is a proteolytic product from the amyloid precursor protein (APP), biomarker of Alzheimer's disease. The outcome of the study suggests that curcumin might break down APP or amyloid-β deposits to Aβ40 which is further released into circulation (Baum et al. 2008; Salehi et al. 2019). Other human trials found no health benefits for Curcumin C3 Complex® and Biocurcumax™, bioavailable curcumin formulation, in Alzheimer's disease patients and healthy older adults, respectively (Salehi et al. 2019).

27.5.2.2 Curcumin in Depression

Lopresti et al. (2014) investigated the potential antidepressant effects of oral curcumin in a randomized, double-blind, placebo-controlled study on 56 patients suffering from major depressive disorder. Curcumin was used as BCM-95® (500 mg capsule containing 88% mixture of curcumin, DMC, and BDMC and 7% essential oil from turmeric rhizomes) at 1 g/day for 8 weeks. Clear benefits of curcumin

supplementation were evident after 4 weeks of treatment: several mood-related symptoms were improved according to the Inventory of Depressive Symptomatology self-rated version (IDS-SR_{30}), questionnaire containing 30 items measuring depressive symptoms. Greater efficacy was observed in patients with atypical depression. In a further randomized, double-blind, placebo-controlled clinical study, Lopresti et al. (2015) examined the effects of curcumin supplementation on several biomarkers associated with antidepressant activity. Fifty patients diagnosed with major depressive disorder received curcumin (BCM-95®, 1 g/day, 8 weeks) or placebo. After 8-week curcumin supplementation, urinary thromboxane B2, substance P and cortisol, and baseline plasma endothelin-1 and leptin were elevated, the latter two being associated with significant reductions in IDS-SR_{30} score. Another study on 108 patients showed that curcumin supplementation has a significant influence on inflammatory biomarkers in major depressive disorder. Curcumin was used as curcuminoid mixture (70% curcumin, 20% DMC, 10% BDMC) at a daily dose of 1 g for 6 weeks. Curcumin supplementation had antidepressant effects (reduction of 17-item Hamilton Depression Rating Scale and Montgomery-Asberg Depression Rating Scale scores.). In addition, it reduced salivary cortisol, IL-1β, and TNF-α plasma levels and enhanced brain-derived neurotrophic factor (BDNF) plasma level (Yu et al. 2015; Salehi et al. 2019). There are two clinical trials on the combined effects of curcuminoids and piperine. Esmaily et al. (2015) investigated the efficacy of the combination on depression and anxiety in obese individuals. Thirty obese subjects received the combination (1 g C3 Complex® and 5 mg Biopiperine® daily) or placebo for 30 days. The combination reduced anxiety (reduction in Beck Anxiety Inventory (BAI) mean score) but not depression (no influence on Beck Depression Inventory (BDI) score). Another study was conducted in patients with major depressive disorder and investigated the efficacy of the combination of curcuminoids and piperine as adjuvant to standard antidepressant therapy. A hundred eleven patients received either standard antidepressant therapy plus combination of curcuminoids and piperine (1 g C3 Complex® and 5 mg Bioperine® daily) or standard antidepressant therapy for a period of 6 weeks. Patients receiving curcuminoid-based adjunctive therapy showed a greater reduction in anxiety and depression symptoms (evaluation by Hospital Anxiety and Depression Scale, HADS and Beck Depression Inventory II, BDI-II) (Panahi et al. 2015). Other two clinical trials proved the efficacy of curcumin as adjunctive therapy in depression. Forty patients with a first episode of depression were treated with curcumin (500 mg/day) and antidepressants or placebo and antidepressants for 5 weeks. The ones receiving curcumin showed a more rapid relief of depressive symptoms (evaluation by Clinical Global Impression-Severity Scale, Hamilton Depression Rating Scale, and Montgomery-Asberg Depression Rating Scale) (Bergman et al. 2013; Salehi et al. 2019). Another clinical trial investigated the effects of fluoxetine (20 mg/day) and curcumin (1 g/day), individually and in combination with 60 patients with major depressive disorder (6-week trial). According to Hamilton Depression Rating Scale, 17-item version (HAM-D17), combination of fluoxetine and curcumin was more efficient than fluoxetine or curcumin alone (77.8%, 64.7%, and 62.5% responders in combination, fluoxetine and curcumin groups, respectively) (Sanmukhani et al. 2014).

27.5.3 Curcumin in Cardiovascular System Disorders

Clinical trials assessing the cardioprotective effects of oral curcumin in healthy individuals reported contradictory outcomes. A trial involving ten healthy volunteers found that 7-day administration of a daily dose of 500 mg curcumin decreased serum lipid peroxides (by 33%) and total cholesterol (by 11.63%) while increasing HDL cholesterol (by 29%) (Salehi et al. 2019). On the other hand, a randomized, double-blind trial enrolling 36 healthy volunteers reported no effects on serum lipid profile for 6-month administration of higher daily doses of curcumin (1 and 4 g) (Baum et al. 2007; Salehi et al. 2019). Longvida® Optimized Curcumin, a bioavailable solid lipid curcumin formulation, demonstrated cardioprotective efficacy in healthy middle-aged people. After 4 weeks, a daily dose of 400 mg (80 mg curcumin) affected several markers relevant for cardiovascular health, namely, lowered plasma triglycerides and soluble intercellular adhesion molecule (sICAM), salivary amylase and increased plasma catalase, myeloperoxidase (no increase in C-reactive protein), and nitric oxide and salivary radical scavenging activity. In addition, plasma amyloid-β protein and alanine aminotransferase were found to be reduced (DiSilvestro et al. 2012). Alwi et al. (2008) designed an interventional study, a randomized, double-blind, controlled trial to evaluate the effects of curcumin in patients with acute coronary syndrome. Fifteen patient groups received low, moderate, or high doses of curcumin (15, 30, and 60 mg thrice daily, respectively) or placebo. After 2 months, an increase in HDL cholesterol and a reduction in total cholesterol and LDL cholesterol were detected in low-dose curcumin group. Surprisingly, moderate and high doses of curcumin were less efficient in ameliorating lipid profile. In a similar randomized double-blind, crossover trial, curcumin supplementation (1 g C3 Complex® and 10 mg Bioperine®/day) increased serum Zn/Cu and reduced Cu/Zn in obese patients, both effects supporting a cardioprotective potential (Mohajer et al. 2014).

27.5.4 Curcumin in Arthritis

Several clinical trials have proved the efficacy of curcumin in osteoarthritis and rheumatoid arthritis. In a pilot randomized double-blind, placebo-controlled, parallel-group trial, 40 patients with mild-to-moderate knee osteoarthritis received either a combination of curcuminoids and bioperine (500 mg C3 Complex® and 5 mg Bioperine® thrice daily) or placebo for 6 weeks. Important reductions in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), visual analogue scale (VAS), and Lequesne's pain functional index (LPFI) scores were observed in curcuminoid group. It is worthy to mention that curcuminoids significantly improved the pain and physical function scores (Panahi et al. 2015). Meriva®, a curcumin-phosphatidylcholine phytosome complex, showed clinical efficacy in patients with osteoarthritis. Administration of Meriva® (1 g corresponding to 200 mg curcumin daily, 3 or 8 months) decreased the WOMAC score by more than 50%, improved the Karnofsky Performance Scale Index, and increased the walking distance in the treadmill test. In addition, Meriva® significantly

reduced inflammation biomarkers such as IL-1β, IL-6, soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble CD40 ligand (sCD40L), erythrocyte sedimentation rate (ESR), and C-reactive protein (Belcaro et al. 2010). A double-blind prospective randomized controlled trial evaluated the efficacy of curcumin as adjuvant therapy for diclofenac in patients with primary knee osteoarthritis. Patients (44) received either diclofenac (75 mg/day) with placebo or diclofenac (75 mg/day) with curcumin (1 g/day) for 3 months. The combination of curcumin with diclofenac proved to be more effective in pain reduction and improvement in function in daily life in comparison with diclofenac alone (according to Knee Injury and Osteoarthritis Outcome Score (KOOS), respectively) but with no significant difference between the two groups (Pinsornsak and Niempoog 2012).

A short-term (2 weeks) double-blind, crossover study enrolling 18 patients with rheumatoid arthritis compared the benefits of curcumin (1200 mg daily) with those of phenylbutazone (300 mg daily). The effects of curcumin, particularly with respect to the improvement in morning stiffness, walking time, and joint swelling, were comparable to those of phenylbutazone (Deodhar et al. 1980). Another clinical study (randomized, single-blinded, pilot study) compared the effectiveness of diclofenac sodium, curcumin (used as BCM-95®), and their combination in patients with active rheumatoid arthritis. Three groups of 15 patients each took diclofenac sodium (50 mg daily), curcumin (500 mg daily), or their combination for 8 weeks. Curcumin induced the most significant improvements in disease activity score (DAS) 28 and American College of Rheumatology (ACR) scores. Besides, curcumin induced the highest reduction in C-reactive protein levels (Chandran and Goel 2012). Curcumin proved to be efficient in postoperative inflammation too. Forty-six patients who underwent surgical repair of inguinal hernia and/or hydrocele took curcumin (400 mg), phenylbutazone (100 mg), or placebo thrice daily for 6 days. Curcumin demonstrated similar efficacy with phenylbutazone in reducing the intensity score (TIS) for spermatic cord edema and tenderness and operative site pain and tenderness (84.2% vs. 86% decrease) (Gupta et al. 2013; Salehi et al. 2019). Curcumin supplementation attenuated inflammation in patients with metabolic syndrome and type 2 diabetes mellitus. A randomized, double-blind, placebo-controlled trial evaluated the effects of curcuminoid (Curcumin C3 Complex®)-piperine (Bioperine®) combination (500 and 5 mg, respectively, twice daily for 8 weeks) on oxidative and inflammatory status in patients with metabolic syndrome. Administration of curcuminoid-piperine combination was associated with an increase in superoxide dismutase (SOD) activity and a reduction in serum malondialdehyde and C-reactive protein (Panahi et al. 2015a).

Kertia et al. (2012) investigated the effects of a curcuminoid mixture isolated from *Curcuma domestica* Val. rhizomes, on COX-2 level in synovial fluid of osteoarthritis patients. The study (prospective, randomized open-blinded end-point (PROBE) study) enrolled 80 knee osteoarthritis patients and demonstrated no significant difference in suppressing COX-2 secretion by synovial fluid monocytes between curcuminoid (30 mg thrice daily) and diclofenac sodium (25 mg thrice daily) after 4-week treatment (decreasing score of COX-2 secretion: 0.70 ± 0.51 in curcuminoid group and 0.67 ± 0.45 in diclofenac group).

27.5.5 Curcumin in Type 2 Diabetes Mellitus

In 14 healthy subjects, turmeric powder (6 g, single dose), taken together with a standard 75 g oral glucose tolerance test, increased postprandial serum insulin levels without affecting plasma glucose levels or glycemic index (crossover trial) (Wickenberg et al. 2010). Curcumin was found to prevent the development of type 2 diabetes mellitus in prediabetic patients. In a randomized, double-blind, placebo-controlled clinical trial, 240 prediabetic patients were assigned to either receive curcuminoid extract (75–85% total curcuminoids, 1.5 g daily or placebo). After 9 months, 16.4% of prediabetic patients in placebo group were diagnosed with type 2 diabetes, whereas no prediabetic patient receiving curcuminoid extract developed the disease. Moreover, curcuminoid extract improved the function of β cells, as revealed by higher HOMA-β and lower C-peptide levels in curcuminoid-treated group. In addition, curcuminoid intervention decreased HOMA-IR and increased adiponectin level (Chuengsamarn et al. 2012). Srinivasan was the first to investigate the benefits of turmeric powder in diabetes. He reported that 5 g of turmeric powder decreased blood glucose in one type 2 diabetes mellitus patient (Salehi et al. 2019). Na et al. (2013) performed a 12-week double-blind, placebo-controlled trial on curcumin glucose-lowering effects in type 2 diabetes. One hundred overweight/obese type 2 diabetes mellitus patients were randomly assigned to receive curcuminoid extract (36.06% curcumin, 18.85% DMC, 42.58% BDMC) at a dose of 300 mg daily or placebo for 3 months. Curcuminoid extract significantly decreased fasting blood glucose, HOMA-IR, serum total free fatty acids, and triglycerides and increased lipoprotein lipase activity. Curcumin proved to be efficient as adjuvant therapy in type 2 diabetes mellitus. Co-administration of glyburide, a permeability glycoprotein (P-gp) substrate, with curcumin, a P-gp inhibitor, showed clinical benefits in patients with type 2 diabetes mellitus and hyperlipidemia. In an open-label, randomized, controlled trial enrolling eight type 2 diabetic patients on glyburide therapy (5 mg daily), 10-day co-administration of curcumin (475 mg daily) enhanced the bioavailability of glyburide (significant increase in Area Under first Movement Curre, AUMC) with a consequent decrease in glucose, LDL, VLDL, and triglyceride levels and increase in HDL level (Neerati et al. 2014). Maithili Karpaga Selvi et al. (2015) investigated the effects of co-administration of turmeric powder in type 2 diabetic patients receiving metformin treatment. In brief, 30 patients received metformin treatment (500 mg twice daily), while other 30 patients took metformin (500 mg twice daily) and turmeric powder (2 g equivalent to 46 mg curcumin, daily). In metformin-treated patients, turmeric supplementation decreased fasting glucose, glycated hemoglobin A1c (HbA1c), lipid peroxidation, LDL cholesterol, non-HDL cholesterol, LDL/HDL ratio, and high-sensitivity C-reactive protein and enhanced total antioxidant status. Rahimi et al. (2016) reported the results of a 3-month randomized, double-blind, placebo-controlled clinical trial evaluating the effects of nano-curcumin on HbA1c, fast blood glucose, and lipid profile in type 2 diabetic patients. Seventy type 2 diabetic patients took nano-curcumin (80 mg curcumin in the form of nano-micelle daily) or placebo. Nano-curcumin decreased HbA1c, fasting blood glucose, triglycerides, and body mass index.

27.5.6 Curcumin in Obesity

Other trials evaluated the effects of curcuminoids in combination with bioperine on markers associated with cardiovascular risk in obesity. In a randomized, double-blind trial with a 2-arm 2-period crossover design, 30 obese patients received either a daily dose of 1 g of curcuminoids (500 mg C3 Complex® and 5 mg Bioperine®) or placebo for 30 days. After a 2-week washout period, the patients were crossed over to alternate treatment for other 30 days. At the end of each treatment period, pro-oxidant-antioxidant balance and antibody titers to markers of oxidative stress such as heat shock protein 27 (Hsp27) and oxidized LDL (oxLDL) were evaluated. Curcumin supplementation resulted in a significant decrease in pro-oxidant-antioxidant balance; no influence on antibody titers to Hsp27 and oxLDL was observed (Sahebkar et al. 2013). Surprisingly, 4-week supplementation with turmeric powder at culinary levels (2.8 g daily corresponding to approx. 112 mg curcumin) did not improve oxidative stress (F2-isoprostanes, oxLDL), inflammation (C-reactive protein, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ), and global metabolic parameters in 62 overweight/obese females with systemic inflammation (randomized, double-blind, placebo-controlled, cross-over study) (Nieman et al. 2012). Ganjali et al. (2014) conducted a randomized, double-blind, crossover trial to investigate the effects of curcumin on serum cytokines in obese subjects. Thirty obese individuals were given curcumin as C3 complex (500 mg) plus bioperine (5 mg) or placebo twice daily for 4 weeks. Curcumin significantly reduced the serum levels of IL-1β, IL-4, and vascular endothelial growth factor (VEGF). Di Pierro et al. (2015) investigated the effects of a bioavailable curcumin formulation (curcumin-phospholipid phytosome) on the weight and omental adipose tissue in overweight individuals with metabolic syndrome. The phytosomal formulation of curcumin consisted of a turmeric extract (95% curcumin) complexed with sunflower phospholipids (20% phosphatidylserine). Twenty-two subjects received phytosomal formulation of curcumin in combination with piperine (800 and 8 mg twice daily), while other 22 subjects received pure phosphatidylserine. After 1 month, the phytosomal formulation of curcumin enhanced weight loss from 1.88% to 4.91%, body fat reduction from 0.70% to 8.43%, waistline reduction from 2.36% to 4.14%, hip circumference reduction from 0.74% to 2.51%, and body mass index reduction from 2.10% to 6.43%. Phosphatidylserine showed no statistical significant effects, most probably due to low dose and/or short administration time.

27.5.7 Curcumin in Gastrointestinal Disorders

27.5.7.1 Curcumin in Peptic Ulcer

Two clinical trials found beneficial effects of turmeric powder in peptic ulcer. Ulcer reduction was reported in 60 ulcer patients after 6–12-week administration of 1 g turmeric powder daily (Salehi et al. 2019). Higher doses of turmeric powder (3 g daily) produced total ulcer remission in 12, 18, and 19 of 25 patients diagnosed

with gastric and duodenal ulcers after 4, 8, and 12 weeks of treatment, respectively. In 20 patients with erosions, gastritis, and dyspepsia, 3 g of turmeric powder daily reduced abdominal pain and discomfort after 1–2 weeks (Prucksunand et al. 2001).

27.5.7.2 Curcumin in *Helicobacter Pylori* Infection

Curcumin alone (700 mg turmeric tablet containing 40 mg curcumin, thrice daily, 4 weeks) showed limited anti-*Helicobacter pylori* activity in comparison with omeprazole-based triple regimen (20 mg omeprazole, 1 g amoxicillin, and 800 mg metronidazole, twice daily for 1 week); the eradication rate in curcumin treatment group was only 5.9% in comparison with 78.9% in omeprazole-amoxicillin-metronidazole treatment group (Koosirirat et al. 2010). A curcumin-based non-antibiotic therapy demonstrated positive therapeutic effects with respect to dyspepsia and gastric inflammation in patients with *Helicobacter pylori* infection. Twenty-five *Helicobacter pylori*-positive patients with functional dyspepsia received curcumin (30 mg) combined with lactoferrin (100 mg), N-acetylcysteine (600 mg), and pantoprazole (20 mg) twice daily for 7 days. Lactoferrin, N-acetylcysteine, and pantoprazole have been reported to possess anti-*Helicobacter pylori* effects. Lactoferrin exerts both bacteriostatic (binding and sequestration of iron that are essential for bacterial growth) and bactericidal (damage of the outer membrane of gram-negative bacteria) effects. N-acetylcysteine, a well-known mucolytic agent, decreases the thickness of the gastric mucus layer thus impairing the growth of *Helicobacter pylori* and improving the mucosal penetration of the other components in the combination. Pantoprazole, a proton-pump inhibitor, creates a hostile environment for *Helicobacter pylori.* Two months after treatment has ended, *Helicobacter pylori* infection was eradicated in 3 of 25 patients; the severity of dyspeptic symptoms (epigastric pain, postprandial fullness, bloating, belching, nausea) and the levels of serum pepsinogen I and II were significantly decreased in all patients (Di Mario et al. 2007).

27.5.7.3 Curcumin in Inflammatory Bowel Disease

Holt et al. (2005) investigated the effects of curcumin in ulcerative proctitis and Crohn's disease in an open-label pilot study. Five patients with ulcerative proctitis or proctosigmoiditis were treated with 550 mg of curcumin twice daily for 1 month followed by 550 mg thrice daily for another month. The authors reported a decrease in indices of inflammation (erythrocyte sedimentation rate, C-reactive protein) to normal levels. In the same study, five Crohn's disease patients were treated with 360 mg of curcumin thrice daily for one month followed by 360 mg of curcumin four times daily for other two months. The treatment reduced the Crohn's Disease Activity Index (CDAI), erythrocyte sedimentation rate, and C-reactive protein levels. Co-administration of curcumin improved the efficacy of medication used to treat ulcerative colitis. Hanai et al. (2006) performed a randomized, multi-center, double-blind, placebo-controlled trial to investigate the effect of curcumin as adjunctive therapy in quiescent ulcerative colitis. Eighty-nine patients were assigned to receive curcumin (1 g twice daily) or placebo in combination with sulfasalazine or mesalamine for 6 months. Curcumin reduced recurrence rate, clinical

activity index (CAI), and endoscopic index (EI) (Salehi et al. 2019). Another multicenter, randomized, double-blind, placebo-controlled trial, designed by Lang et al. (2015), enrolled 50 patients with active mild-to-moderate ulcerative colitis treated with mesalamine. Patients did not respond to maximal mesalamine oral (4 g/day) and local (1 g in 4 g enema or 1 g suppository/day) therapy administered for 2 weeks. Curcumin (3 g/day) or placebo was added to patients receiving mesalamine. After 1 month, significant clinical and endoscopic remissions were detected in curcumin group (53.8% clinical remission, 65.3% clinical response, 38% endoscopic remission vs. 0% clinical remission, 12.5% clinical response, and 0% endoscopic emission in placebo group, respectively).

27.5.8 Curcumin in Liver Disorders

Rahmani et al. (2016) investigated the effects of curcumin on liver fat content and biochemical and anthropometric indices in patients with nonalcoholic fatty liver disease. Eighty patients were enrolled in a randomized, double-blind, placebo-controlled study and received amorphous dispersion of curcumin formulation (500 mg daily equivalent to 70 mg of curcumin) or placebo for 8 weeks. It was found that curcumin administration significantly reduced liver fat content, serum levels of total cholesterol, LDL, triglycerides, aspartate aminotransferase, alanine aminotransferase, fasting blood sugar, glycated hemoglobin, and also body mass index. Echogenicity of the liver, as revealed by ultrasonography, was improved in 78.9% of patients in curcumin group but only in 27.5% of patients in placebo group. In addition, liver fat content showed no increase in curcumin group but increased in 17.5% of patients in placebo group. Panahi et al. (2017) reported the efficacy of Meriva® in nonalcoholic fatty liver disease. A hundred and two patients were randomly assigned to receive Meriva® (500 mg twice daily) or placebo for 8 weeks. Meriva® reduced body mass index, waist circumference, aspartate aminotransferase, and alanine aminotransferase levels. Ultrasonography showed improvement in liver echogenicity in 75% patients in Meriva® group and only 4.7% patients in placebo group. The study of Kim et al. (2013) reported hepatoprotective effects for fermented turmeric. Fermentation of crushed turmeric was carried out in the presence of *Aspergillus oryzae* (2%) at 25 °C for 36 h followed by drying and standardization to 0.79 mg curcumin per 1 g powder. A randomized, double-blind, placebo-controlled trial enrolled 60 subjects with mild-to-moderate elevated alanine aminotransferase levels (40–200 IU/L). The subjects took either fermented turmeric powder (3 g daily) or placebo. After 12 weeks, serum alanine aminotransferase and aspartate aminotransferase levels were significantly reduced in subjects receiving fermented turmeric powder.

27.5.9 Curcumin in Gall Bladder Disorders

Curcumin showed cholekinetic effects in healthy individuals. Two studies (randomized, double-blind, placebo-controlled, crossover and randomized, single-blind, III

phase, crossover studies) investigated the effects of different doses of curcumin (20, 40, or 80 mg, single dose) on gall-bladder contraction at different intervals (0.5, 1, 1.5, and 2 h) after administration. The 40 mg dosage was found to induce 50% contraction of gall bladder at 2 h after administration (Salehi et al. 2019). With respect to biliary dyskinesia, the effects of curcumin alone have not been investigated yet. Only a combination preparation containing dried extracts of greater celandine (*Chelidonium majus* L.) and turmeric was found to cause a faster reduction in dumpy and colicky pain in patients with biliary dyskinesia after 1-week treatment (Salehi et al. 2019).

27.5.10 Curcumin in Pancreatitis

Curcumin in combination with piperine has been reported to be efficient in pancreatitis. Twenty patients with tropical pancreatitis were randomized to receive curcumin (500 mg/day) with piperine (5 mg/day) or placebo. After 6 weeks, significant reduction in erythrocyte malonyldialdehyde and increase in erythrocyte glutathione (both markers of oxidative stress) were detected in group treated with curcumin and piperine (Durgaprasad et al. 2005; Salehi et al. 2019).

27.6 Application in Food (Including Correctly Cooking Foods Rich in Curcumin)

Curcumin (E 100) is authorized as food additive (food color) in the European Union with maximum permitted levels (MPLs) of 50–500 mg/kg in foods and at *quantum satis* in some food categories (EFSA 2014). As main component of turmeric powder which is widely used as food flavoring, coloring, and preservative agent, curcumin is an ingredient of many dishes worldwide. Heat treatment is known to affect the chemical integrity and consequently the health benefits of many food ingredients (vitamins, proteins, lipids, carbohydrates) (Suresh et al. 2009). Therefore, the impact of different types of cooking on the stability and bioactivity of curcumin is of high interest. The instability of curcumin during heating can be mainly attributed to its diketone moiety which is very susceptible to hydrolysis. In addition to hydrolysis into smaller compounds, conjugated double-bond shift and polymerization might also occur during heat treatment (domestic cooking) (Suresh et al. 2009).

Different cooking methods affect the bioactivity of curcumin. For example, heating was found to increase the water solubility and bioactivity of curcumin. Heating curcumin in water (1 or 5 mg curcumin/mL water at 90 °C with further heating for 10 min in a boiling water bath) increased solubility 12-fold without affecting its stability (Kurien et al. 2007). Moreover, heat-solubilized curcumin showed antioxidant activity (80% reduction in 4-hydroxynonenal-mediated oxidation of proteins) (Kurien et al. 2007) and immunomodulating effects (43–52% reduction in antibody-antigen interaction) (Kurien et al. 2010). On the other hand, heating curcumin in aqueous solution (40 μM) for 11 and 15 min at 95 °C caused

curcumin degradation (67 and 82%, respectively) with changes in antioxidant activity such as decrease in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and increase in 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging effects at 11 min, increase in nitrite scavenging effect at 15 min, and increase in 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) radical scavenging effects at 10 and 15 min heating. Microwave heating was found to cause time and radiation power-dependent degradation and changes in antioxidant activity of aqueous curcumin. Exposure to microwave radiation at 500 W for 5 min degraded almost 70% of curcumin. However, the microwave heating at 500 W for 5 min caused more pronounced enhancements (46–100%) in ABTS, nitrite, and AAPH radicals scavenging activity in comparison with regular heating (Jung et al. 2016). Ten min heat treatment in a pressure cooker (15 psi) decomposed curcumin into smaller compounds such as vanillin, vanillic, and ferulic acids (Suresh et al. 2009). Typical Indian cooking (heating spices, including turmeric in hot oil) also enhances the health benefits of curcumin. In order to mimic the effects of traditional cooking, curcumin was pyrolyzed with coconut fat or olive oil (5 mg curcumin and 50 mg fat were pyrolyzed at 250 °C for 20 min). The process generated more hydrophilic products and isoforms (DMC, iso-DMC, BDMC, iso-BDMC, iso-curcumin) but also diketene curcumin (1,5-bis (4-hydroxy-5-methoxyphenyl)-1,4-pentadiene-3-one), previously reported as a synthetic curcumin analogue; the latter showed higher stability and higher anti-tumor activity (induction of apoptosis and G2 arrest in B78H1 melanoma cells) than curcumin (Dahmke et al. 2014).

To conclude, domestic cooking affects curcumin structure, but structure modification is not always accompanied by a reduction in bioactivity; traditional Indian cooking, microwave heating, and even regular heating enhanced different biological effects (antioxidant, antitumor) of curcumin.

27.7 Safety: Toxicity and Side Effects

Curcumin possesses a good safety profile. The US Food and Drug Administration (FDA) has approved curcumin as *generally recognized as safe* (GRAS) (Gupta et al. 2013). The Joint United Nations and World Health Organization Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) reported that the allowable daily intake (ADI) value of curcumin is 0–3 mg/kg body weight (Hewlings and Kalman 2017).

Curcumin proved to be safe and well tolerated even at high doses. Escalating single doses of C^3 Complex™ (75% curcumin, 23% DMC, 2% BDMC) from 0.5 to 12 g showed an excellent tolerance in healthy volunteers with minimal adverse events (diarrhea, headache, rash, yellow stool) in 30% of subjects (Lao et al. 2006). Long-term high-dose curcumin administration was also well tolerated. In a 3-month phase I clinical trial in patients with high-risk or premalignant lesions, oral curcumin showed no toxicity in humans in doses up to 8 g/day (Cheng et al. 2001). Most of clinical studies reported no significant

toxicity for curcumin or curcumin-based preparations. Only few studies indicated adverse effects for high doses of curcumin. The most frequently reported side effects were gastrointestinal events (abdominal pain, reflux, flatulence, diarrhea, nausea) (Gupta et al. 2013; Fadus et al. 2017). The highest patient compliance was found for daily doses of 2–4 g, most side effects occurring at daily doses higher than 4 g (Fadus et al. 2017). A good tolerability was also found for highly bioavailable curcumin Theracurmin (180 mg curcumin/day for 8 weeks in patients with knee osteoarthritis) (Nakagawa et al. 2014).

27.8 Marketed Products

Curcumin is widely marketed as capsules, tablets, energy drinks, ointments, and cosmetics (Gupta et al. 2013). The most common conventional but also bioavailable curcumin-based formulations (registered and unregistered trademarks) are listed in Table 1; for the latter, the relative bioavailabilities compared to conventional

Table 1 Conventional and bioavailable curcumin-based formulations

Product	Description	Relative bioavailability compared to conventional curcumin
Biocurcumax™ (BCM®)	Turmeric powder and turmeric essential oil (45% ar-turmerone)	6.9
Cavacurmin®	γ-cyclodextrin-based formulation (approx. 15% total curcuminoids)	85
Curcumin C3 Complex®	Curcuminoids (450 mg of curcumin, 40 mg of DMC, 10 mg of BDMC)	–
Curcumin C3 Complex® + Bioperine	Curcuminoids and bioperine	20
CurcuWin®	Water-soluble formulation containing 20–28% turmeric extract	136.3
CurQfen™	Formulation containing 40% curcumin and fenugreek soluble dietary fibers	15.8
LongVida®	Solid lipid curcumin particle-based formulation (20–30% curcumin)	100
Meriva®	Curcumin-phosphatidylcholine phytosome complex (18–20% curcuminoids)	48
MicroActive curcumin	Micronized formulation (25% curcuminoids)	9.7
Micronized curcumin	Micronized powder (25% curcumin powder)	9
NovaSol®	Liquid micelles containing 7% curcumin powder (equiv. to 6% curcumin)	185
Theracurmin®	Colloidal-nanoparticle-based formulation containing 10% curcumin and 2% other curcuminoids	15.9

(unformulated) curcumin are also given (Jamwal 2018; Sharma et al. 2004). The safety and efficacy of most of them have been evaluated in clinical studies as previously described in Sects. 5 and 7.

Curcumin has a low oral bioavailability, and therefore numerous approaches have been developed to overcome this limitation. Reduction in particle size of curcumin by micronization improved dissolution and hence absorption. Co-administration with bioavailability enhancers such as piperine (alkaloid in black pepper) increased curcumin bioavailability 20-fold. Piperine is a potent inhibitor of P-glycoprotein (P-gp), a transporter that limits the absorption of curcumin by effluxing it back into the intestinal lumen. Additionally, piperine inhibits UGTs, enzymes involved in the phase II metabolism of curcumin thus enhancing the circulating level of free curcumin (Jamwal 2018). Turmeric essential oil also acts as a bioavailability enhancer. One of its constituents, α-turmerone, was reported to significantly inhibit P-gp (Yue et al. 2012). Curcumin aqueous solubility and absorption were improved via inclusion complexes with cyclodextrins. A better stability and diffusion rate across the phospholipid bilayer of cell membranes was achieved by phytosomal formulation of curcumin (complexation of curcumin with phosphatidylcholine). The use of soluble dietary fibers, such as the ones from fenugreek, protected curcumin against degradation and provided a prolonged release with enhanced absorption. Other formulations (water-soluble formulation with 63–75% polyvinylpyrrolidone as hydrophilic carrier, micelles with 93% Tween 80 as surfactant, solid lipid particles prepared with soy lecithin, docosahexaenoic acid, stearic acid, and vitamin C) increased curcumin bioavailability over 100-fold compared to conventional curcumin (Jamwal 2018).

27.9 Patents

Numerous patents have been developed related to strategies to enhance the bioavailability of curcumin and its use against different diseases; recent ones are listed in Table 2.

27.10 Perspectives

Curcumin is undoubtedly a phytochemical with a huge therapeutic potential and good safety profile. Its low oral bioavailability considerably limits its use in health promotion, disease prevention, and treatment. Therefore, as many clinical trials showed, administration of highly bioavailable curcumin-based formulations is a prerequisite for beneficial effects in humans. Development of formulations that enhance curcumin absorption rate and stability and provide a sustained or targeted delivery of curcumin are effective strategies to improve therapeutic efficacy of curcumin and open new perspectives for its clinical valorization.

Table 2 Patents related to curcumin

Patent	Title	Ref.
US9861677B2	Composition to enhance the bioavailability of curcumin	Antony (2018)
US10004687B2	Liposomal curcumin for treatment of diseases	Kurzrock et al. (2018)
WO2016013026A1	Curcumin-sophorolipid complex	Singh et al. (2016)
WO2016167732A1	Development of curcumin and piperine-loaded double-layered biopolymer-based nano delivery systems by using electrospray/coating method	Sezgin (2016)
WO2016140904A1	Method to solubilize curcuminoids in water	Majeed and Nagabhushanam (2016)
WO2016113762A1	Modified cyclodextrin coated magnetite nanoparticles for targeted delivery of hydrophobic drugs	Joy and Naduvilidam (2016)
US20160101066A1	Fibrin wafer/disk as a biological carrier for sustained delivery of curcumin	Krishnan and Sreedharam (2016)
WO2015081319A2	Activity enhancing curcumin compositions and methods of use	Banerjee et al. (2015)
WO2015025263A1	A novel composition of curcumin with enhanced bioavailability	Gopi (2015)
US9192644B2	Bioavailable curcuminoid formulations for treating Alzheimer's disease and other age-related disorders	Frautschy and Cole (2015)
US9170257B2	Method and system for measuring the pharmacokinetics of liposomal curcumin and its metabolite tetrahydrocurcumin	Helson (2015)
WO2014036534A1	Curcumin-ER, a liposomal PLGA sustained release nano-curcumin for minimizing QT prolongation for cancer therapy	Ranjan et al. (2014)
WO2014145851A1	Product and method for improving bioavailability of therapeutic compounds	Petersen et al. (2014)
WO2014068597A2	Formulation of curcumin with enhanced bioavailability of curcumin and method of preparation and treatment thereof	Kuriakose (2014)
WO2012125830A2	Curcumin combination with anti-type 2 diabetic drugs for prevention and treatment of disease sequelae, drug-related adverse reactions, and improved glycemic control	Helson (2012)
WO2011101859A1	A novel water-soluble curcumin-loaded nanoparticulate system for cancer therapy	Sanjeeb and Chandana (2011)
US7968115B2	Liposomal curcumin for treatment of cancer	Kurzrock et al. (2011)
US20110257126A1	Water-soluble curcumin compositions for use in anticancer and anti-inflammatory therapy	Neven et al. (2011)
WO2008131059A2	Intranasally administering curcumin in a bolus of helium gas to treat Alzheimer's disease	Dimauro (2008)
WO2007143635A1	Method to prepare pure curcumin	Kim (2007)

27.11 Cross-References

► Antioxidants in Diets and Food
► Introduction of Phytonutrients

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Isothiocyanates in Food

28

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Contents

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Abstract

Isothiocyanate is an enzymatic hydrolysate of glucosinolates that are abundant in Brassicaceae or Cruciferae crops such as broccoli, radish, and cabbage. In addition to application in food products, isothiocyanates can effectively prevent DNA damage and cancer caused by various carcinogens in the diet including polycyclic aromatic hydrocarbons, heterocyclic amines, and nitrosamines. Moreover, isothiocyanates have antioxidant, anti-inflammatory, and antibacterial properties. In this chapter, the source, bioavailability, metabolism, bioactivity, toxicity, and application of isothiocyanates will be summarized. In addition, recent developments in isothiocyanate applications and future research needs will be discussed, to serve as a reference for industries, scientists, and consumers.

Keywords

Isothiocyanate · Metabolism · Bioactivity · Application · Safety · Product

28.1 Introduction of Isothiocyanates

28.1.1 Source of Isothiocyanates

Isothiocyanate is a class of small molecular compounds with the structure of –N=C=S and abundant in Brassicaceae or Cruciferae vegetables (Table 1). These compounds are formed in a bioconversion progress with glucosinolates as the substrate and catalyzed with β-thioglucosidase (EC 3.2.1.147) (also known as myrosinase). In addition, isothiocyanate can be artificially synthesized when used as food additive or essential oil.

Common Brassicaceae or Cruciferae vegetables that contain glucosinolates include *Arabidopsis thaliana*, broccoli, cabbage, white radish, kale, mustard, and radish.

28.1.2 Classification of Isothiocyanates

The classification of isothiocyanates depends on the types of their substrates – glucosinolates that have three different types according to their structure: aliphatic, terpenoids, and aromatic compounds. Isothiocyanates produced after enzymatic conversion are also divided into aliphatic, terpenoids, and aromatic groups. Common classifications are as follows: allyl isothiocyanate, benzyl isothiocyanate, phenethyl isothiocyanate, indole-3-carbinol, sulforaphane, iberin, sulforaphene, and erucin (Table 2). They exhibit different chemical structures; allyl isothiocyanate is an aliphatic compound, whereas both benzyl isothiocyanate and phenethyl isothiocyanate are aromatic and contain a benzene ring. Structural differences lead to differences in lipophilicity and hydrophilicity. Moreover, they have different potentials for inhibiting microorganisms, anti-oxidation, and anticancer activities.

Table 1 List of glucosinolates found in different Brassica vegetables

Structure of R-group	Semisystematic names of R-groups	Trivial names	*Brassica* spp. (+ present; −absent)				
			Cabbage	Brussels sprouts	Cauliflower	Broccoli	Chinese cabbage
$CH_2 = CH–CH_2–$	Allyl	Sinigrin	+	+	+	+	−
$CH_2 = CH–CH_2–CH_2–$	But-3-enyl	Gluconapin	+	+	+	+	+
$CH_2 = CH–CH_2–CH_2–CH_2–$	Pent-4-enyl	Glucobrassicanapin	−	−	+	+	+
$CH_2 = CH–CH–CH_2–$	(2R)-2-Hydroxybut-3-enyl	Progoitrin	+	+	+	+	+
$CH_3–S–CHOH–CH_2–CH_2–$	3-Methylthiopropyl	Glucoibervirin	+	−	+	−	−
$CH_3–S–CH_2–CH_2–CH_2–CH_2–$	4-Methylthiobutyl	Glucoerucin	+	+	+	+	−
$CH_3–SO–CH_2–CH_2–CH_2–$	3-Methylsulphinylpropyl	Glucoiberin	+	+	+	+	−
$CH_3–SO–CH_2–CH_2–CH_2–CH_2–$	4-Methylsulphinylbutyl	Glucoraphanin	+	+	+	+	+
$CH_3–SO–CH_2–CH_2–CH_2–CH_2–CH_2–$	5-Methylsulphinylpentyl	Glucoalyssin	−	−	−	−	+
$CH_3–SO_2–CH_2–CH_2–CH_2–CH_2–$	4-Methylsulphonylbutyl	Glucoerysolin	+	−	−	−	−

Table 2 Chemical structure of isothiocyanates

Common name	Side chain name	Side chain structure	MW	Main dietary source
Aliphatic ITC				
Allyl ITC	2-Propenyl	$CH_2 = CH–CH_2^-$	99	Cabbage and horseradish
Erucin	4-Methylthiobutyl	$CH_3–S–(CH_2)_4^-$	161	Turnip and kohlrabi
Hexyl ITC	Hexyl	$CH_3(CH_2)_3^-$	143	
Iberin	3-Methylsulfinylpropyl	$CH_3–SO–(CH_2)_3^-$	163	Broccoli and cabbage
Sulforaphane	4-Methylsulfinylbutyl	$CH_3–SO–(CH_2)_4^-$	177	Broccoli
Sulforaphene	4-Methylsulfinyl-3-butenyl	$CH_3–SO–CH=CH–(CH_2)_2^-$	175	Radish
Aromatic ITC				
Benzyl ITC	Benzyl	$C_6H_5–CH_2^-$	149	Wasabi and mustard
Phenyl ITC	Phenyl	$C_6H_5^-$	135	
Phenylethyl ITC	2-Phenylethyl	$C_6H_5–(CH_2)_2^-$	163	Watercress
Indolyl ITC				
Indole-3-carbinol	1H-Indol-3-yl-methanol	$C_8H_6N–CH_2OH$	147	All vegetables

28.2 Bioavailability and Metabolism

The bioavailability and metabolism of isothiocyanates are closely related to glucosinolates. Cruciferous plants contain both glucosinolates and myrosinase, and when the tissue is destructed (e.g., masticate), glucosinolates are hydrolyzed to form isothiocyanates. Glucosinolate is decomposed by the plant myrosinase in the small intestine or by the bacterial myrosinase in the colon. Metabolites of isothiocyanates can be detected in human urine 2–3 h after the consumption of cruciferous plant. Studies show that a small portion of the intact glucosinolates can be absorbed and excreted intact, although the absorption mechanism is unclear (passive diffusion or facilitated transport, Fig. 1) (Sã¸Rensen et al. 2016). Understanding the bioavailability, transport, and metabolism of isothiocyanates is fundamental to understanding their mechanisms of protective functions.

28.2.1 Metabolic Pathway

The human body mainly ingests isothiocyanate by eating glucosinolate-containing cruciferous vegetables. Glucosinolate is a secondary metabolite of cruciferous plants

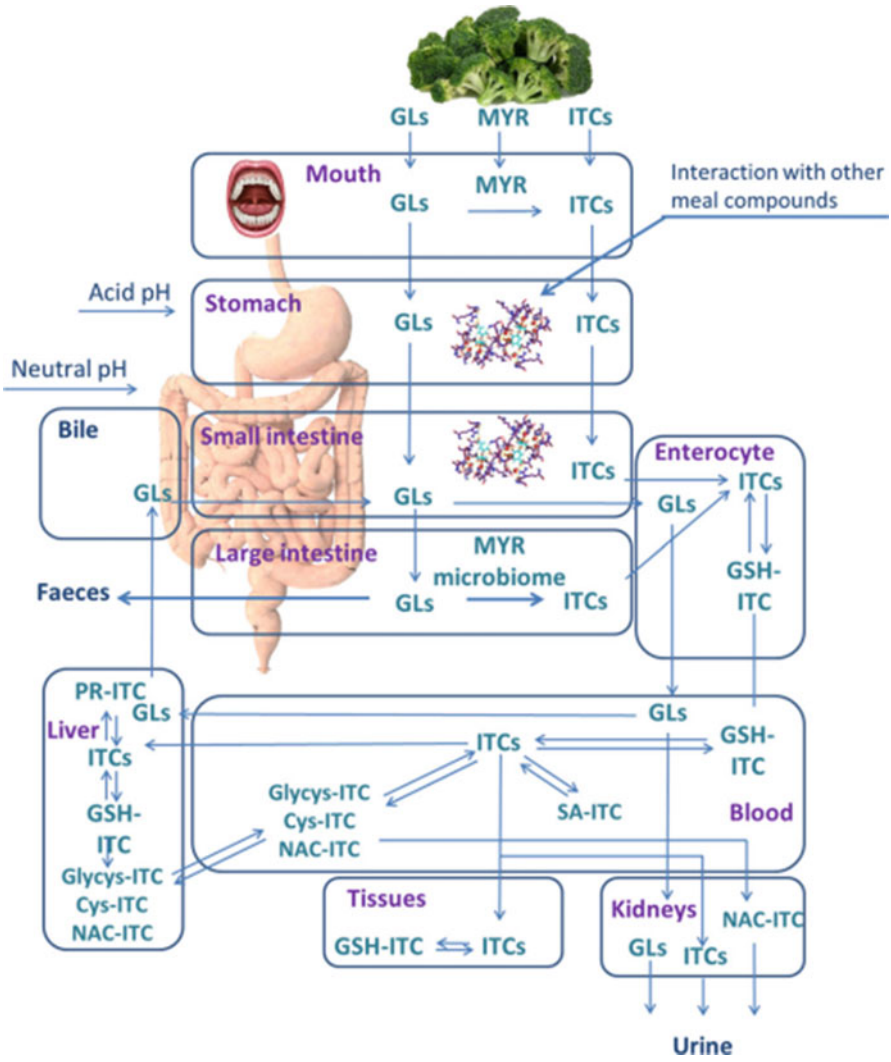

Fig. 1 Overview of the metabolic fates of glucosinolates (GLs) and isothiocyanates (ITCs)

located in the vacuole of plants. When plant tissue is subjected to external damage such as chewing, glucosinolates in the vacuole (as shown in Fig. 2) undergo enzymatic reaction catalyzed by cytoplasm myrosinase to produce flavor substances with a hot and spicy odor. The main three major flavors include isothiocyanates, thiocyanates, and nitriles, as shown in Figure 3. The stomach and intestinal bacteria in the human body also contain myrosinase that can degrade glucosinolates as well (Tian et al. 2018).

Isothiocyanate is a substrate for glutathione transferase. The enzyme-catalyzed nucleophilic attack of the sulfhydryl group of glutathione on the central carbon of the

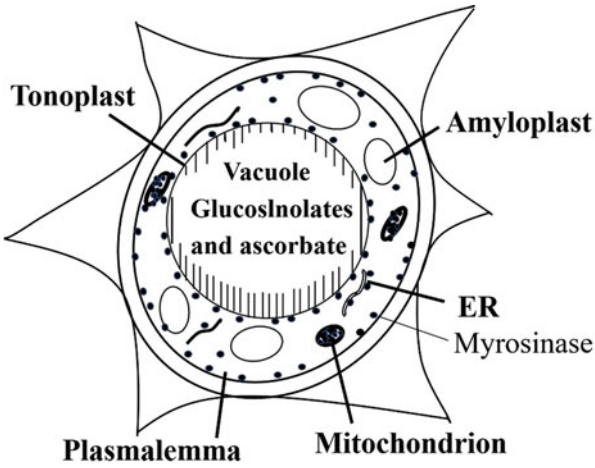

Fig. 2 A model for compartmentation of myrosinase and glucosinolates

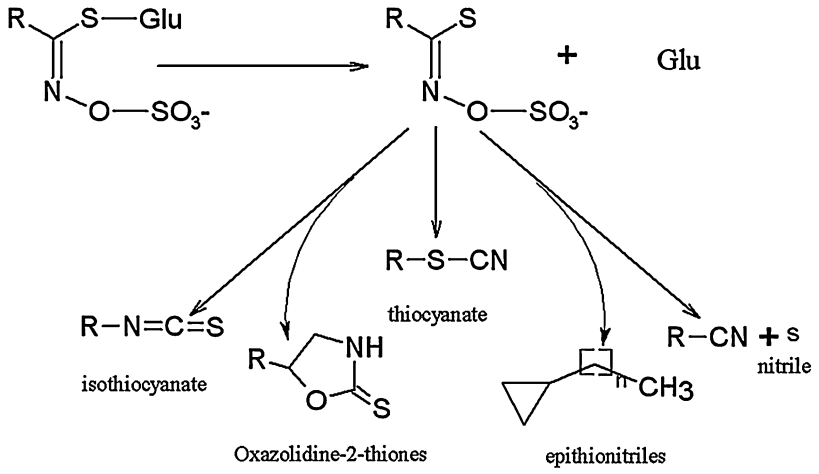

Fig. 3 Summary of metabolic pathways for glucosinolate degradation

isothiocyanate group results in the formation of glutathione dithiocarbamates that are modified by a sequence of enzymatic reactions, leading ultimately to the formation of *N*-acetylcysteine dithiocarbamates (also known as mercapturic acids). This progress is usually discharged in the urine.

Oral or intestinal formed isothiocyanate is primarily metabolized by the thiol uric acid pathway. Enzymatic initiation upon initial conjugation with glutathione to produce dithiocarbamic acid, such as *N*-acetylcysteine-, cysteine-, and cysteinyl glycine-isothiocyanate conjugates (Ye et al. 2002).

28.2.2 Bioavailability

Bioavailability of isothiocyanates can be assessed by measuring metabolites in blood and urine. Allyl isothiocyanate absorption in the intestine is fast and complete. Allyl isothiocyanate is mainly excreted in the urine in rats and mice. In one study, urinary excretion of radiolabeled allyl isothiocyanate was up to 80% of total dose in rats and mice (within 72 h), and the amount of allyl isothiocyanate excreted by feces was approximately 3–12% (for rats) and 4–9% (for mice) of the total dose after oral administration (Ioannou et al. 1984). In addition to renal excretion, allyl isothiocyanate can be excreted through breathing, and a small amount of allyl isothiocyanate-derived radioactive carbon dioxide is detected in animal exhalation.

Like allyl isothiocyanate, urine seems to be the main route of excretion for benzyl isothiocyanate. Within 72 h, exhaled excretion was minimal (0.4%), followed by feces (5.6%), and most was urine (92%); less than 0.5% remained in the carcasses, which is much less than that reported for allyl isothiocyanate in rats. The isothiocyanate metabolized by the enterohepatic circulation is negligible because it accounts for only 4%.

After oral administration in male rats, phenethyl isothiocyanate was rapidly absorbed into the blood. The cumulative excretion in urine and feces within 48 h was 7% and 47%, respectively, of which about 15% was excreted with feces; 14% was exhaled as carbon dioxide, and 0.007% was exhaled as an alcohol soluble organic compound.

28.2.3 Factors Affecting the Bioavailability and Metabolism of Isothiocyanates

28.2.3.1 Vegetable Types and Varieties

The bioavailability of isothiocyanate is directly proportional to the amount of glucosamine hydrolyzed, and the more glucosinolate content is ingested, the greater the corresponding isothiocyanate content. There are significant differences in the content of glucosinolates in different cruciferous vegetables (broccoli, Brussels sprouts, cabbage, broccoli, kale) (Yen and Wei 2010). He et al. studied the traditional Chinese cruciferous Brassica vegetables and found that the difference was also great (He et al. 2005). The total amount of glucosinolate in the cabbage was the greatest, and the amount of bok choy (green vegetables) consumed in the south part of China was the lowest.

28.2.3.2 Oral Phase: Mastication

When chewing vegetables begins, the myrosinase activates and induces the hydrolyzation of glucosin. During chewing, the vegetable tissue is crushed and ground to mix the released compound with the saliva in the food mass. The formation of isothiocyanate during chewing depends on the chewing strength; the

concentrations of glucosinolates, myrosinase, and all factors could affect the velocity of reaction.

28.2.3.3 pH

pH can affect the degradation of glucosinolates. The results showed that the initial content of glucosinolates decreased to 88–97% at pH range of 3.6–9.1, while the retention rate decreased when the pH was less than 3.6, leaving only 60% at pH 1.5 (Jing et al. 2012). The pH values of the human stomach and intestine are 2 and 7.8, respectively, and it was reported that the glucosinolates in cauliflower were reduced to 69% in the stomach. Then they were reduced by 12% in the intestines. However, the myrosinase of the plant itself has been denatured under acidic conditions in the stomach, but glucosinolates can be decomposed by microorganisms in the intestine (Sã¸Rensen et al. 2016). Little is known about the stability of the isothiocyanates during digestion.

28.2.3.4 Microbes

Microorganisms in the intestine can degrade glucosinolates from cruciferous vegetables to produce isothiocyanates. It is generally believed that glucosinolates reach the large intestine before they are decomposed (Elfoul et al. 2001). Glucosides cannot be degraded by alpha-amylase and pepsinase in the upper intestine nor by intestinal trypsin in vitro (Lai et al. 2010). In contrast, glucosinolates can be hydrolyzed by intestinal microbial communities. At the same time, intestinal microflora is the main source of myrosinase in vivo. For instance, Llanos et al. (1995) screened three strains that could degrade the glucosinolate by measuring the decomposition of allyl glucosinolates by 42 lactic acid bacteria. *Lactobacillus agilis* had the strongest decomposition ability and mainly produced lactic acid and allyl isothiocyanate.

28.3 Bioactivities of Isothiocyanates

Isothiocyanates may prevent DNA damage and cancer caused by several dietary carcinogens (Barnett et al. 2015). The mechanism may be that isothiocyanates can detoxify and accelerate the excretion of carcinogens by inhibiting the activity of phase I reductase and inducing the production of phase II enzymes. In addition, isothiocyanates can also prevent diseases. Evidence has shown that isothiocyanates bring to bear their activity through a variety of unique but interrelated signaling pathways, including those involved into detoxification, inflammation, and epigenetic regulation. The effects of isothiocyanates on these pathways are mainly regulating cellular antioxidant systems, enzyme induction or inhibition, and selective gene expression, interferencing with cell cycle and signaling pathways and effects on tumor microenvironment, and inducting of apoptosis or autophagy (Nakamura and Miyoshi 2010). These activities will be demonstrated with examples in the following paragraphs (Fig. 4).

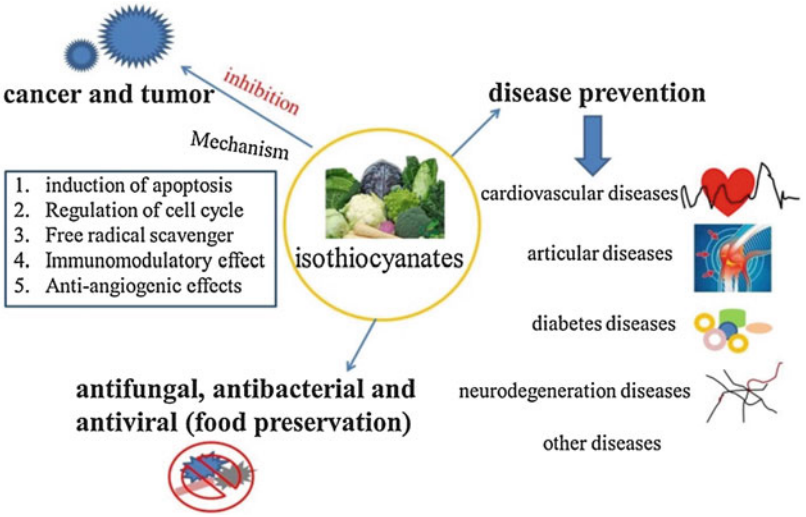

Fig. 4 Bioactive activities of isothiocyanates

28.3.1 Inhibition of Cancer and Tumor Activity

Epidemiological studies showed that the consumption of cruciferous vegetables (including broccoli) could reduce the risk of inducing certain cancers. Isothiocyanate is the most important anticancer and antitumor active ingredients in this vegetable and has certain preventive ability against cancer (Latté et al. 2011; Herr et al. 2010). It has been reported that the per capita intake of glucosinolate in Canada is estimated to be about 16 mg/day. The corresponding intakes in the UK and Japan are estimated to be 30 mg/day and 112 mg/day, respectively. The American population is much smaller. The intake of the Chinese population has not been known. Sepkovic et al. provided isothiocyanates (6–7 mg/kg) to young healthy people every day in 1994 (Sepkovic et al. 1994). The trial lasted for 3 months. The results showed that oral isothiocyanate intake was well tolerated and no adverse reaction was observed in people. The dose can be greater than 1000 mg/kg (fw). Also, it was a strong inducer of cytochrome P4501A isoenzymes and a chemical carcinogen inhibitor in some animal models (Shiizaki et al. 2017).

28.3.1.1 Induction of Apoptosis

Apoptosis, also known as programmed cell death, has been identified as one of the strategies to address cancer cell growth. Isothiocyanates can directly induce apoptosis through mitochondrial release of cytochrome and activation of apoptotic protease activator 1, caspase-9, and adenosine triphosphate. It has been found that the stimulatory effect of isothiocyanates is the proteolytic cleavage of polymerase and the appearance of caspase activity followed by induction of apoptosis in cervical cancer and PC-3 prostate cancer cells.

Phenethyl isothiocyanate modulates different mitochondrial proteins such as B-cell lymphoma-2, Bid, and Bax leading to the release of cytochrome C in the cytoplasm, which is the intrinsic mechanism of apoptosis. Allyl isothiocyanate is considered to be an effective method because of the high bioavailability in urine after ingestion. It caused cell cycle arrest, then it increased apoptotic rate, and lower concentrations of allyl isothiocyanate can induce genotoxicity in mutant cells of the TP53 gene. In addition, the apoptotic effect of sulforaphane is mediated by regulation of acetylated histones and subsequent upregulation of p21 and Bax proteins (Sávio et al. 2014). Studies have reported that isothiocyanate-induced apoptosis is inhibited by inhibition of proteasome activity and tumor suppressor p53 is significantly accumulated in multiple myeloma cells, independent of reactive oxygen species production. These results suggest that isothiocyanates may initiate apoptosis through several pathways. In addition, there is evidence that the cytotoxic effects of isothiocyanates may selectively target cancer rather than normal cell types.

There are some testimonies suggested that phenethyl isothiocyanate, benzyl isothiocyanate, and phenyl isothiocyanate inhibited lung tumorigenesis and O^6-methylguanine formation (DNA-adduct formation in the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumors) in the DNA of lung cells from A/J mice which were treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Morse et al. 1991; Morse et al. 1989) In recent years, sulforaphane and phenethyl isothiocyanate have significant effects on proliferation and apoptosis of human lung adenocarcinoma cell line LLTE-A2, which caused G(2)/M arrest in a time and dose-dependent manner($p < 0.05$). On the other hand, it increased apoptotic cell fraction ($P < 0.05$) to prevent (Liang and Lai 2008). Besides, phenylhexyl isothiocyanate has also been found to prevent cancer by blocking the initiation of carcinogen-induced tumors in rodents (Nishikawa et al. 2010).

28.3.1.2 Regulation of Cell Cycle

Numerous studies have shown that isothiocyanates can interfere with the cell cycle progression of cancer cells and the effects of isothiocyanates on cell growth and cell cycle progression in HeLa cells by flow cytometry. Cell accumulation at the G2/M phase interface was observed 16 h after treatment with 10 mM allyl isothiocyanate, 2.5 mM benzyl isothiocyanate, or 2.5 mM phenethyl isothiocyanate, the concentration of which inhibited cell growth by 41–79% of control. These results indicate that isothiocyanates delay the cell cycle progression of HeLa cells, leading to inhibition of cell growth (Hasegawa et al. 1993).

Isothiocyanates have been reported to regulate the expressions of protein kinases (cyclin and cyclin-dependent kinases) involved in cell cycle progression. Isothiocyanates mediated the suspension of drug-resistant cells in G1 and G2/M phase in a dose and time-dependent manner. Isothiocyanates mediated G2/M phase inhibition of cell cycle which has been studied in PC-3 cells by downregulating the accumulation of Cdk1, cell division cycle 25C, and Tyr-15 phosphorylation (inactive) Cdk1. The antiproliferative effect of isothiocyanates was also investigated in colorectal adenocarcinoma cells by upregulating phosphorylation of p21 and enhanced checkpoint kinase 2, followed by sustained G2/M arrest. The results

showed that cell which was exposed to benzyl isothiocyanate leads to inhibition of G2/M progression and upregulation of G2/M cell cycle arrest regulatory genes (including p21). The essential point is the regulation of cell cycle progression. In the G2/M phase, a prerequisite for the apoptosis induced by benzyl isothiocyanate is mediated by p38 mitogen-activated protein kinase.

Furthermore, it has been suggested that transcriptional regulation of 14-3-3σ, Cdc25C, and GADD45 by p21 and p53 mediates cell cycle arrest. Inducing cell cycle arrest is considered a promising chemopreventive strategy.

28.3.1.3 Free Radical Scavenger

Oxidative stress is defined as the imbalance between the levels of reactive oxygen species produced and the ability of biological systems to eliminate or to quench the active intermediates formed (Sita et al. 2016), which is the basis of the pathogenesis of various diseases. Isothiocyanates are a class of phytochemicals with good cancer prevention potential and its double-edged swords that regulate cellular oxidative stress (Zhang et al. 2005). Exposure of cells to isothiocyanates could increase their ability to resist oxidative damage. During the process of 12-O-tetradecanoylphorbol-13-acetate-induced oxidative rupture of HeLa 60 cells, benzyl isothiocyanate and other isothiocyanates were potent inhibitors for a large number of superoxide anions, and the mechanism of biologically active antioxidant activity of benzyl isothiocyanate was mainly implemented by the Keap1/NF-E2-related factor 2/antioxidant response element approach. Gülçin found in in vitro antioxidant experiments that broccoli isothiocyanates extract had strong free radical scavenging ability, which was similar to the antioxidant capacity of ascorbic acid, but the temperature and storage stability of isothiocyanates extract were better than that of ascorbic acid (Gülçin et al. 2004).

In addition, isothiocyanates regulate different phase II enzymes. Phase I enzymes (cytochrome P450 enzymes) generally increase the reactivity of fat-soluble compounds and produce some reactive molecules that may be more toxic than the parent molecule. Phase II enzymes (glutathione-S-transferase, aldehyde reductase, S-methyltransferase, *N*-acetyltransferase, etc.) increase water solubility and promote excretion of these metabolites from the body. Therefore, inhibition of phase I and induction of phase II enzymes are essential for protecting cells from DNA damage from carcinogens and reactive oxygen species (Vig et al. 2009). For example, phenethyl isothiocyanate partially induces the expression of phase II enzymes mediated by antioxidant response elements via c-Jun N-terminal kinase-1 and nuclear factor erythrocyte-2-related factor 2 (Keum et al. 2003). Allyl isothiocyanate, benzyl isothiocyanate, and 6-(methylsulfinyl)hexyl isothiocyanate have the ability to induce phase II and antioxidant enzymes via the nuclear factor erythrocyte-2-related factor 2-dependent signal transduction pathway (Mizuno et al. 2011). As a result, activation of the nuclear factor erythrocyte-2-related factor 2 pathway by isothiocyanates confers protection against carcinogens and has emerged as a promising strategy for cancer prevention. Currently, epidemiological evidence supports the role of cruciferous vegetables against lung, colorectal, breast, prostate c, and pancreatic cancers (Herr 2010).

In addition, isothiocyanate can increase the antioxidant capacity of animal cells by inducing the activity of phase II enzymes or by increasing the level of glutathione. Benzyl isothiocyanate induces expression of GST isoenzyme to mediate intracellular reactive oxygen intermediate in a short time (1 h), which is sufficient to increase glutathione S-transferase activity. Allyl isothiocyanate and phenyl isothiocyanate inhibit tumor-specific angiogenesis by significantly reducing the production of nitric oxide and tumor necrosis factor-α (Thejass and Kuttan 2007).

28.3.1.4 Immunomodulatory Effect

The tumor microenvironment is some resemblance to inflammatory lesions and plays a very important role in the process of carcinogenesis. Inflammation encourages cancer cells to grow in a bimodal manner. Some studies have shown that phenethyl isothiocyanate can directly regulate the inflammatory process. Phenethyl isothiocyanate has been shown to inhibit chemically induced inflammatory responses in mice. Mechanistic studies have shown that inflammatory mediators such as nitric oxide, tumor necrosis factor-α, and interleukin-10 are inhibited in the LPS-stimulated macrophages and macrophage migration inhibitory factor can be inhibited by covalent modification of phenethyl isothiocyanate.

A few reports also linked immunomodulatory effects to the anticancer activity of phenethyl isothiocyanate. For example, phenethyl isothiocyanate has been shown to protect mouse liver and lung from changes caused by cigarette smoke. Bioinformatic analysis of phenethyl isothiocyanate-treated animal tumors revealed regulation of genes involved in the inflammation and extracellular matrix pathways. In conclusion, these findings indicate that anti-inflammatory effects contribute to the anticancer effects of phenethyl isothiocyanate (Gupta et al. 2014).

Other studies have found that allyl isothiocyanate exhibits potent anti-inflammatory activity in macrophages cultured in vitro but has little anti-inflammatory activity in mice in vivo. The anti-inflammatory effects of allyl isothiocyanate were accompanied by an increase in the nuclear translocation of nuclear factor erythrocyte-2-related factor 2 and increased the mRNA and protein levels of the nuclear factor erythrocyte-2-related factor 2 target gene heme oxygenase 1 (Wagner et al. 2012).

Unlike allyl isothiocyanate, the anti-inflammatory properties of sulforaphane have been well-documented. Tumor necrosis factor-mediated NF-κB activation in THP1 human monocytes were inhibited by sulforaphane. In *H. pylori*-infected mice, sulforaphane treatment resulted in a reduction in symptoms of gastritis (Wagner et al. 2012). Also, Brandenburg et al. reported through the results of their researches showed the anti-inflammatory potential of sulforaphane in the brain. An established model of neuroinflammation was used in the assay based on stimulation of microglia with endotoxin lipopolysaccharides to determine the effect of sulforaphane on lipopolysaccharides-induced microglia expression of certain cytokines.

28.3.1.5 Anti-angiogenic Effects

In addition to their effects on carcinogen metabolism and cancer cell proliferation, isothiocyanate has been shown to effectively interfere with angiogenesis in vitro and in vivo. In vitro studies, allyl isothiocyanate showed anti-angiogenic and pro-

apoptotic effects, inhibited the growth of ascites tumors and angiogenesis, which may play a potential role in the potential chemoprevention and chemotherapeutic activity of isothiocyanate (Cavell et al. 2011).

28.3.2 Disease Prevention

There has been increasing interest in the field of chemoprevention using natural remedies in recent years. Organic isothiocyanates, mainly allyl isothiocyanate, benzyl isothiocyanate, phenylethyl isothiocyanate, sulforaphane, erucic acid, and phenylhexyl isothiocyanate, are widely studied chemopreventive agents. The isothiocyanates present in many dietary cruciferous vegetables have been shown to have preventive and therapeutic activity against several chronic human degenerative diseases, including cancer, cardiovascular diseases, neurodegeneration, and diabetes. There is also growing evidence that isothiocyanates can inhibit cardiovascular diseases and neurodegenerative diseases. The chemical preventive activity of isothiocyanate has traditionally been attributed to its ability to alter the metabolism of carcinogens. During the first stage of metabolism, the polarity of the toxin increases due to reduction, oxidation, or hydrolysis reactions. If the polarity is strong enough, the metabolites produced will be expelled.

28.3.2.1 Prevention of Cardiovascular Diseases

Cardiovascular diseases like myocardial ischemia-reperfusion injury, atherosclerosis, and coronary heart disease are among the leading causes of death in the world. There are some well-known cardiovascular risk factors, such as hypertension, hypercholesterolemia, smoking, obesity, diabetes, and ageing. Oxidative stress is related to all these known cardiovascular risk factors and the process of forming atherosclerotic plaques. These observations emphasize that oxidative stress is the contact between cardiovascular risk factors and vascular disease and therefore is the goal of cardiovascular prevention.

Sulforaphane has been shown to increase in cell viability and decrease in DNA fragmentation of neonatal cardiomyocytes which might be interceded by multiple antioxidant proteins expression and reactive oxygen species production. Also, the activity of antioxidants and phase II enzymes was induced by sulforaphane in rat aortic smooth muscle cells and in mitochondria of aortic smooth muscle cells which is isolated like catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, quinone oxidoreductase-1, and glutathione in vivo (Leoncini et al. 2011). Studies on isolated rat hearts have shown that the protective influence of sulforaphane on cardiac ischemia-reperfusion injury can be attributed to the inhibition of the increase left ventricular end-diastolic pressure after ischemia. Sulforaphane also improved pressure coronary blood flow in the development of left ventricle after ischemia and reduced lactate dehydrogenase levels during reperfusion.

Isothiocyanates have the cardioprotective activity against ischemia/reperfusion injury and were confirmed in an in vivo model. To prove it, the isolated hearts were

subjected to ischemia/reperfusion injury after rats were fed broccoli for 30 days (Mukherjee et al. 2009). Broccoli improved post-ischemic ventricular function while decreased myocardial infarct size and cardiomyocyte apoptosis. Bahadoran et al. (Bahadoran et al. 2012) demonstrated that supplementation with 10 g/day broccoli sprouts had beneficial effects on the most typical diabetic dyslipidemia and lipid-related parameters in type 2 diabetic patients for 4 weeks. Overall, isothiocyanates in vivo and clinical studies of cardiovascular disease protection provide hopeful results.

28.3.2.2 Prevention of Articular Diseases

Chondrocyte death is very important in cartilage degradation and progression of joint diseases involving osteoarthritis and rheumatoid arthritis. Recently, it has been showed that treatment of osteoarthritis chondrocytes with sulforaphane decreased the production of various matrix metalloproteinases and the reduction of Jun N-terminal kinase activation by the pro-inflammatory cytokines interleukin-1 and tumor necrosis factor-α. In chondrocyte lines, sulforaphane also enhanced quinone oxidoreductase-1 activity and glutathione levels. Treatment of grown human chondrocyte monolayer cultures with sulforaphane reduced cell death which was induced and reduced caspase activity which is effector and initiator by tumor necrosis factor-α plus cycloheximide. In addition, in the in vitro and in vivo models of osteoarthritis, sulforaphane prevented cartilage destruction in cells (Davidson et al. 2017).

28.3.2.3 Diabetes Prevention

Type 1 diabetes characterized by destruction of β-cells and lack of b-cell mass during inflammatory isletitis which is a T-cell-driven autoimmune disease. Sulforaphane treats a rat pancreatic β-cell line which prevented interleukin-1b and interferon-gamma-induced b-cell damage. The effect of the activation of the nuclear factor erythrocyte-2-related factor 2 pathway was mediated. The results of the recovery of normal insulin secretion response of glucose in cytokine-treated rat islets and the developmental blockade of type 1 diabetes which in streptozotocin-treated mice were confirmed (Song et al. 2008). Other studies have found that severe inflammation of the animals is treated with cyclophosphamide alone and dark-colored urinary bladders are normalized in morphological analysis by allyl isothiocyanate or phenyl isothiocyanate treatment (Manesh and Kuttan 2005; Zhu et al. 2008). Sulforaphane improved kidney performance and minimized pathological changes in the glomerulus and improved motor nerve conduction velocity, blood flow, and pain behavior in a mouse model of streptozotocin-induced diabetes (Zheng et al. 2011). These findings have promoted the potential use of isothiocyanates in alleviating metabolic disorders and prevent kidney damage and diabetes-related pain (Ye et al. 2002).

28.3.2.4 Protective Effects Against Neurodegeneration

The reason for slow development of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis slowly developing is that oxidative stress and neuroinflammation are actively

involved in neuronal death. Reactive oxygen species can directly oxidize and damage macromolecules like DNA, proteins, and lipids, culminating in neurodegeneration at the end (Calabrese et al. 2007).

Isothiocyanates show neuroprotective activity or neurodegeneration in vitro or in vivo models, respectively. They can directly scavenge free radicals or indirectly increase endogenous cellular antioxidant to defenses by activating the nuclear factor erythrocyte-2-related factor 2 transcription factor pathway (Giacoppo et al. 2015). These preventive effects may be primarily due to their unique ability to activate the nuclear factor erythrocyte-2-related factor 2/antioxidant response element pathway. An alternative mechanism of action for neuroprotection is regulation of the signal transduction cascade or its effect on gene expression. Therefore, isothiocyanates could prevent and treat neurodegenerative diseases that were thought as a promising source of alternative medicine.

Sulforaphane protects primary cortical neurons from damage induced by dopamine oxidation products, which are capable of forming adducts with cellular thiols. Therefore, isothiocyanates could prevent and treat neurodegenerative diseases which would be seen as a promising source of alternative medicine (Spencer et al. 2010).

28.3.2.5 Prevention of Other Diseases

Continued exposure to cigarette smoke or contamination can interfere with the lung cleansing, and macrophages drive a powerful innate immune defense system, which leads to chronic obstructive pulmonary disease. In patients and mouse models of the disease, sulforaphane adjusted this pathway and increased the ability of lung macrophages to sequester and inactivate *Haemophilus influenzae* and *Pseudomonas aeruginosa*, both of which exacerbate chronic obstructive pulmonary disease (Fimognari et al. 2012).

Steatosis is the result of abnormal liver function and whose character is the accumulation of triglycerides in hepatocytes. In addition, sulforaphane can increase the levels of quinone oxidoreductase-1 and heme oxygenase-1 mRNA. Sulforaphane can reduce LXRα-induced hepatic lipogenesis, which may represent the result of nuclear factor erythrocyte-2-related factor 2 activation, because quinone oxidoreductase-1 and heme oxygenase-1 are transcribed from the Nrf2 target gene (Kay et al. 2011).

Sulforaphane has a protective effect on cyclosporin-induced gingival fibrosis and the phototoxic effects induced by UVB irradiation. In addition, sulforaphane can increase the expression level of quinone oxidoreductase-1 and heme oxygenase-1 mRNA.

The ultraviolet light of the sun is strong carcinogen that can strengthen inflammation, burn the skin, and cause skin cancer. Activation of nuclear factor erythrocyte-2-related factor 2 by sulforaphane protects body to alleviate UVB-induced inflammation and sunburn responses.

A recent study showed that sulforaphane can be used in conditions that require immunosuppression, involving autoimmune diseases and transplant diseases. The genetic basis of these pathologies involves the role of interleukin-2 and related T-cell activations. Sulforaphane obviously inhibited T-cell proliferation of primary human

T lymphocyte cultures activated by anti-CD3 and anti-CD28. At the same time, sulforaphane also reduced the production of interleukin-2, suggesting its role in proliferation inhibition.

28.3.3 Antifungal, Antibacterial, and Antiviral Inhibiting Effects

Isothiocyanates exhibit biological activity against a variety of pathogens which from food, including viruses (Mochida and Ogawa 2010), fungi (Smolinska and Horbowicz 1999), bacteria (Miran et al. 2010). It was studied the toxic effects of aliphatic isothiocyanate and aromatic isothiocyanate on growth parameters of different fungi, such as sclerotia viability, sclerotia growth and germination of *Sclerotinia sclerotiorum*. The result showed that allyl, methyl, and benzyl isothiocyanates were the most fungicidal of the tested compounds under the experimental conditions. Troncoso-Rojas et al. (2005) tested the effect of benzyl isothiocyanate on the growth of *Alternaria alternata*. The results of in vitro experiments showed that the minimum inhibitory concentration of benzyl isothiocyanate is 0.1 mg/mL, which indicated that benzyl isothiocyanate could control black spot disease in tomato fruit and had no adverse effects on postharvest quality of tomato. Kim and Lee (Kim and Lee 2010) tested the inhibitory effects of phenyl isothiocyanate and its derivatives on intestinal bacteria growth. The results showed that phenyl isothiocyanate (mg/disk) strongly inhibited the growth of *C. difficile* and *Clostridium perfringens* and moderately inhibited the growth of *E. coli* at a dose of 6.0 mg/day.

The length of the isothiocyanate hydrocarbon chain and the size of the molecule, whether it has a double bond thiol group and a thiol group, affect their antimicrobial activity. The side chains of isothiocyanate are derived from different amino acids, and these differences confer different biological properties (Sotelo et al. 2015). Isothiocyanates containing sulfonium groups are the most effective growth inhibitors for oral pathogens, followed by aromatic isothiocyanates (containing benzene rings) and aliphatic isothiocyanates. The presence of double bond in the chemical structure of the isothiocyanates appears to increase antimicrobial activity. For example, the sulfonyl group (CH_3–SO–CH=CH–CH_2–CH_2–) is structurally similar to sulforaphane (CH_3–SO–CH_2–CH_2–CH_2–CH_2–) but has a double bond, which has lower antibacterial activity against *Candida albicans*. Isothiocyanates containing thiol (-S-) or sulfinyl (-SO-) groups have different antimicrobial activities compared to other isothiocyanates. Glucosinolate (CH_3–S–CH_2–CH_2–CH_2–CH_2–) is structurally similar to sulforaphane (CH_3–SO–CH_2–CH_2–CH_2–CH_2–) (having a sulfinyl group) but has a thiol group and exhibits higher antimicrobial activity against *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. Antimicrobial activity also depends on the length of the hydrocarbon chain. Short-chain benzyl isothiocyanate (C_6H_5–CH_2–) has a stronger antimicrobial potential than phenylethyl isothiocyanate (C_6H_5–CH_2–CH_2–) against *Candida albicans*, *Streptococcus mutans*, *L. casei*, and *Staphylococcus aureus* (Li et al. 2013).

28.4 Application and Processing Effects of Isothiocyanates in Food

Isothiocyanates are used in foods and seasonings such as mustard oil, mustard sauce, green mustard, kimchi, and sauce because of their typical pungent and spicy taste. Isothiocyanates also have a bacteriostatic effect and can be used for preservation of foods. In addition, their anticancer and anticancer properties can make them be used as functional ingredients of health foods. Pure isothiocyanates added to foods are generally chemically synthesized rather than directly extracted from vegetables. It is worth noting that the amount of isothiocyanates in the food are affected by the cooking way.

28.4.1 Application of Isothiocyanates in Food

28.4.1.1 Food Flavor Compounds

Isothiocyanates have a spicy pungent odor and are the main volatile components in mustard and horseradish sauce, usually used in salads and seafood. As early as 1959, allyl isothiocyanate was used as a food additive. Currently, there are 19 isothiocyanate-based fragrance compounds approved by the Flavor and Extract Manufacturers Association in the United States (Kong et al. 2011). There are 15 isothiocyanate-based fragrances in the list of synthetic fragrances for foods permitted in GB 2760-2014 (Standards for the Use of Food Additives). The characteristics and sources of these natural spices are shown in Table 3.

As a food flavor, the study of the physical and chemical properties of isothiocyanate is of great significance. Isothiocyanates can chemically react with water molecules and hydroxides in neutral and alkaline solutions. Citric acid sugar esters and salad oils can increase the stability of isothiocyanates in some degree. The degradation rate at 37 °C is significantly higher than 0 °C. Therefore, isothiocyanates should be stored in a low temperature and acidic environment. Since isothiocyanates can pass through some food packaging materials such as polyethylene and polypropylene, corrosion-resistant packaging materials should be selected to reduce loss of food flavor and contamination to other foods during storage of isothiocyanate-containing products.

28.4.1.2 Food Preservative

Isothiocyanates have a broad spectrum of antibacterial properties and can be used as a food preservative in food preservation and packaging. Studies have shown that at the concentration of 0.04 mol/L, benzyl isothiocyanate, 3-methylthiopropyl isothiocyanate, phenylpropyl isothiocyanate, butyl isothiocyanate, and 4-pentenyl isothiocyanate have significant inhibition on the growth of *Vibrio parahaemolyticus* and *Salmonella*. At the same time, benzyl isothiocyanate has a good inhibitory effect on two Gram-positive pathogens, such as *Staphylococcus aureus* and *Listeria monocytogenes* (Wang et al. 2017). The inhibitory effects of the same concentration of allyl isothiocyanate on the growth of different microorganisms were as follows,

Table 3 Structural formula, aroma characteristics, and natural presence of currently used isothiocyanate perfumes

Name	FEMA number	GB 2760 Number code	Aroma characteristics (Burdock and Burdock 2002)	Natural sources (Caboni et al. 2013; Afsharypuor and Suleimany 2002; Radonić et al. 2010; Afsharypuor and Sepehrnejad 2006)
Methyl isothiocyanate	4426	–	Pungent smell like mustard and horseradish	Garlic, wasabi, wine
Ethyl isothiocyanate	4420	–	Pungent smell like mustard and garlic	Wasabi, mustard
Isopropyl isothiocyanate	4425	S1090	Pungent mustard-like smell	Mustard, capers fruit, cabbage, wasabi
Allyl isothiocyanate	2034	S0762	Mustard-like strong spicy smell	Onions, cabbage, horseradish, Milk, cauliflower, Brussels sprouts, wasabi, and cruciferous plants' seeds
Butyl isothiocyanate	4082	–	Wasabi, horseradish, mustard-like spikes	Mustard, wasabi, broccoli, capers fruits
Sec-butyl isothiocyanate	4419	S1085	Green incense	Mustard, radish, wasabi
Isobutyl isothiocyanate	4424	S1086	Green incense	Cabbage, garlic, capers fruit
3-Butenyl isothiocyanate	4418	S1079	Strong irritating odor, strong osmotic	Cabbage, milk, cauliflower, mustard, horseradish, wasabi
Amyl isothiocyanate	4417	S1089	Spiked green incense	Radish, kale seeds
Isoamyl isothiocyanate	4423	S1091	Spiked green incense	Radish seeds, kale seeds
Hexyl isothiocyanate	4422	–	Spiked green incense	Radish, horseradish
5-Hexenyl isothiocyanate	4421	S1081	Strong irritating scent	Radish, horseradish, wasabi
3-(Methylthio) propyl isothiocyanate	3312	S0899	Radish, horseradish, sulfur, and mud-like smell	Cabbage, cauliflower, horseradish, mustard head
4-(Methylthio) butyl isothiocyanate	4414	–	Cabbage, radish-like aroma	Cabbage, Brussels sprouts, broccoli
5-(Methylthio) pentyl isothiocyanate	4416	S1088	Radish-like aroma	Radish, horseradish

(continued)

Table 3 (continued)

Name	FEMA number	GB 2760 Number code	Aroma characteristics (Burdock and Burdock 2002)	Natural sources (Caboni et al. 2013; Afsharypuor and Suleimany 2002; Radonić et al. 2010; Afsharypuor and Sepehrnejad 2006)
6-(Methylthio) hexyl isothiocyanate	4415	S1087	Radish-like aroma	Horseradish
Benzyl isothiocyanate	4428	S0893	Mild watercress, drug, horseradish, and oily-like flavor	Celery, lone, nasturtium
Phenylethyl isothiocyanate	4014	S1321	Radish, gooseberry, green incense, and sulfur-like smell	Cabbage, cauliflower, broccoli, mustard, wasabi, radish, mushroom, turnip, watercress, spinach

Paecilomyces variabilis > *Saccharomyces cerevisiae* > *Escherichia coli* > *Bacillus subtilis*, and the inhibition rate increased with the increase of allyl isothiocyanate concentration. The antibacterial effect of allyl isothiocyanate fluctuates very little in a pH range from 4.5 to 7.0. The inhibition rate of allyl isothiocyanate is higher than that of potassium sorbate, sodium benzoate, sodium propionate, nisin, and sodium hydrogen sulfite. Under the premise of the same anti-corrosion effect, the addition of allyl isothiocyanate can reduce the sterilization degree of the cabbage. When 0.01% allyl isothiocyanate is used together with potassium sorbate, the dosage of potassium sorbate can be reduced by about 0.05% in "Chongcai" (Niu et al. 2005).

The strong pungent odor of allyl isothiocyanate limits its application to food industry and has not been widely used for the postharvest preservation of fruits and vegetables. It was found that microcapsule technology could overcome the shortcomings of allyl isothiocyanate to reduce its irritating odor and volatility, thereby prolonging the antibacterial time and the shelf life of food. Wu et al. (2015) used gelatin and gum arabic as wall materials and allyl isothiocyanate as core material to make microcapsules, which could effectively control the release of allyl isothiocyanate and reduce the irritating odor to significantly prolong storage time and maintain quality of tomato. Allyl isothiocyanate microcapsules were able to significantly prolong the shelf life of kimchi (Ko et al. 2012). Piercey et al. (2012) found that β-cyclodextrin-allyl isothiocyanate microcapsules could significantly inhibited aerobic bacteria and *Listeria* in fresh onion slices and promoted the preservation of onions.

In addition to the application in microcapsule technology, isothiocyanates can also form molecularly imprinted polymers, which together with chitosan to form a new active antimicrobial packaging material for beef preservation. Compared to low-density polyethylene packaging film, the active allyl isothiocyanate – molecularly imprinted polymers film – can significantly delay muscle degradation (Huang et al. 2018).

28.4.1.3 Functional Food Ingredients

Epidemiological studies reported that cruciferous vegetables can effectively prevent cancer. Long-term consumption of Brassica vegetables can significantly reduce the risk of various cancers such as stomach cancer, prostate cancer, and melanoma (Fahey et al. 2012) and reduce the incidence of lung cancer caused by smoking and air pollution (Tang et al. 2010). This is primarily due to the anticancer property of isothiocyanates (Musk et al. 1995).

The literature showed that 150 g of fresh broccoli may contain 56–112 g of sulforaphane with anticancer and cardiovascular protection. However, pre-treatment will cause most of the loss. For high-risk groups of this type of disease, the daily intake of broccoli does not achieve good preventive effects. The consumption and frequency of consumption are related to its anticancer and anticancer effects. Sulforaphane is an effective immune-enhancing ingredient in broccoli. The daily intake of 34 mg of glucosinolate per body (50 kg) can effectively enhance immunity. The British Food Research Institute believes that eating broccoli two to three times a week may enhance human health. It is recommended to consume about 300 g of broccoli each time. If you choose to eat every day, the daily intake is 70–80 g.

Commercially available sulforaphane capsules extract sulforaphane glucosinolate from broccoli seeds, each containing 30 mg of sulforaphane glucosinolate, which is hydrolyzed to sulforaphane in the body and functions as 20 times broccoli. As a nutritional supplement, sulforaphane capsules are not a substitute for medicines.

28.4.2 Effects of Processing Methods on Isothiocyanates

Isothiocyanates usually have good biological activity, but the properties are very unstable. For example, sulforaphane (1-isothiocyanate-4-methanesulfonylbutane) in broccoli is sensitive to temperature, while it's not sensitive to light (Li et al. 2007). The conversion of glucosinolates to isothiocyanates is influenced by many factors including heating, so cooking of mustard and broccoli may result in loss of sulforaphane (Alvarez-Jubete et al. 2014).

Different cooking methods differed in the loss of isothiocyanates. Kapusta-Duch et al. (2016) studied the changes of glucosinolates concentrations and their degradation products of three vegetables (rutabaga, green cauliflower, and purple cauliflower) during the cooking process. It was found that the total glucosinolates content in three plants decreased by 6.6%, 68.9%, and 69.2%, respectively. The cooking process resulted in an 11.0% reduction of isothiocyanates contents in green cauliflower and 42.4% reduction in purple cauliflower whereas 329.4% increase in rutabaga.

Isothiocyanates can be obtained from thioglucoside hydrolyzed by endogenous glucosidase in broccoli (isothiocyanates). However, during the cooking process, the kale is easily deactivated, so the concentration of isothiocyanates is reduced after cooking. Wu et al. (2018) compared the content of isothiocyanates in raw broccoli, directly fried with broccoli, hydrolyzed, and fried with broccoli. The results showed that the total isothiocyanate, sulforaphane content were the highest in the raw

broccoli group, and were the lowest in the directly fried with broccoli group. The content and activity of isothiocyanates were not significant difference between hydrolyzed and fried with broccoli group and control group. In addition to cooking methods, the type of vegetable oil used in cooking also affects the content of isothiocyanates in broccoli. Moreno et al. (2010) studied the effects of five different edible vegetable oils on total glucosinolates. The results showed that the use of extra virgin olive oil, soybean oil, peanut oil, or safflower oil didn't reduce the concentrations of total glucosinolates in broccoli. Because high temperature heating, oxygen, and salt can accelerate the destruction of isothiocyanates, vegetables should be heated at a low temperature for a short period of time to reduce the oxygen action and protect the isothiocyanates in vegetables as much as possible.

28.5 Safety: Toxicity and Side Effects

Although isothiocyanates are health beneficial components, overdose intake is toxic to humans and animals. Allyl isothiocyanate and derivatives are moderately toxic and are listed as restricted imported materials in many countries. For instance, a small amount of isothiocyanates inhalation can cause bronchial inflammation such as vomiting and coughing. When exposed to a long-term direct contact, it may cause impaired eczema. If the intake concentration is high enough, it may lead to vascular central and respiratory center paralysis and stimulate the digestive organs to cause gastroenteritis and can cause thyroid hypertrophy (Musk et al. 1995).

28.5.1 Acute Toxicity of Isothiocyanates

Related studies have shown that acute oral toxicity of 95% allyl isothiocyanate in Sprague-Dawley rats is moderate poisoning (LD_{50} male and female rats 271 mg/kg) and acute dermal toxicity is low toxic(LD_{50} male and female rats greater than 2000 mg/kg). Acute oral toxicity of 20% allyl isothiocyanate in Sprague-Dawley rats is low toxicity (LD_{50} male and female rats 1470 mg/kg), and acute dermal toxicity to Sprague-Dawley rats is low toxicity (LD_{50} male and female rats greater than 2000 mg/kg) (Wu 2010).

28.5.2 Chronic Toxicity of Isothiocyanates

Long-term exposure to isothiocyanates can also lead to chronic toxicity. Some structures of side-chain groups of isothiocyanates have β-thiol glucosinolates which are unstable with enzymatic hydrolysis of isothiocyanates and are automatically cyclized in polar solutions. Allyl isothiocyanate is generally considered to be a typical goiters factor to make goiter in animals (Borek et al. 1995). The mechanism of toxicity is that it competes with iodine, which reduces the absorption of iodine and causes swelling of the thyroid gland. 5-Hexenyl isothiocyanate is the primary flavor

component of wasabi. Akagi et al. (2018) performed a subchronic toxicity study on 5-hexenyl isothiocyanate at a daily dose of 0, 3, 12, and 48 mg/kg body weight for 6 weeks of male and female rats for 13 weeks; 5-hexenyl isothiocyanate-targeted organs were identified as the bladder, heart, and liver. Heart weight increased in male 48 mg/kg body weight group and female over 12 mg/kg body weight group. Male and female 12 mg/kg body weight group had bladder hyperplasia, and female 48 mg/kg body weight showed nodular hyperplasia. Male and female rats had no adverse reactions in 3 mg/kg body weight group.

28.5.3 Genotoxic Effects of Isothiocyanates

Numerous studies have shown that high doses of isothiocyanate are genotoxic, leading to sister chromosome exchange and chromosomal aberrations, DNA damage, and carcinogenesis in mammalian cells. Kassie and Knasmüller (2000) confirmed the genotoxicity of allyl isothiocyanate and phenyl isothiocyanate by *E. coli*, *Salmonella* TA100/TA98, and human Hep G2 cell experiments. At high doses of 90–270 mg/kg, allyl isothiocyanate showed moderate genotoxicity in mouse tissues, while phenyl isothiocyanate had no significant toxic effect in all mouse tissues. Bovine serum albumin and human saliva were found to significantly reduce the genotoxicity of isothiocyanates.

Murata et al. (2000) found that high concentrations of allyl isothiocyanate, phenyl isothiocyanate, and benzyl isothiocyanate (2.5–10 mol/L) could cause DNA damage and product 8-oxodG (marker of DNA oxidative damage) in the presence of Cu^{2+}. Laser imaging densitometry tests confirmed that the point mutation was located at the 273 ACG codon of the P53 tumor suppressor gene. Sequence changes at this site would result in the loss of normal biological activity of P53. However, in the absence of Cu^{2+}, isothiocyanates did not cause DNA damage. In human myeloid leukemia HL-60 cells, allyl isothiocyanate could induce the production of 8-oxodG.

28.6 Marketed Products of Isothiocyanates

With the improvement of people's consumption level and the emphasis on food safety, research and development of high-efficiency, nontoxic, residue-free pest control measures and food preservation methods have received widespread attention. In 1937, Walker reported the rapeseed of the genus cruciferous, together with the degradation products of isothiocyanate like sulfur mustard. Antibacterial activity and broad-spectrum fungicides, including allyl isothiocyanate and allyl isothiocyanate are the main product solutions active against many plant pathogenic fungi, bacteria, worms, and viruses. Some human pathogenic bacteria such as *Escherichia coli* bacteria, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Helicobacter pylori*, and so on are also biologically active. Isothiocyanate can effectively control pathogens and reduce the decay of fruits, vegetables, and meat during storage. However, since it is highly irritating and difficult to store directly, it is made into an allyl isothiocyanate microcapsule, which is convenient to use. According to the selection

conditions of the microcapsules, the encapsulation efficiency of the microcapsules can reach more than 90%, which can effectively control the release of allyl isothiocyanate and reduce the irritation. The results showed that the tomato microcapsules had a longer preservation time and the tomato sensory quality was better. Meat preservation has always been a serious problem, especially in terms of fresh meat preservation. The isothiocyanate microcapsules can reduce the loss rate of the gravy, delay the increase of the pH value and reduce the redness, and better maintain the original taste and elasticity of the gravy.

In daily life, isothiocyanates are widely used as a food additive with their unique spicy flavor. For example, allyl isothiocyanate is considered to be the main active ingredient of horseradish and is often used for seasoning of cold dishes, raw seafood, and the like. In addition to being used as a seasoning, allyl isothiocyanate is widely used as a food preservative. Adding 0.0001% allyl isothiocyanate in juice, the quality of the juice is still in very high standard even after 1 year of storage in the store. Therefore, more and more researchers examined the antibacterial effect of allyl isothiocyanate and tried to apply it to food packaging.

In addition to its antibacterial action, isothiocyanate also has an antiseptic effect, such as antiseptic effect on chitosan smears during storage of aquatic products. The film-forming ability of the raw material is prepared by the composite coating process of the antibacterial activity of the carp. The results show that the allyl isothiocyanate chitosan composite coating is better than the allyl isothiocyanate chitosan coating and the control group. The allyl isothiocyanate chitosan composite coating can effectively inhibit the bacterial colony. At the same time, it can slow down the fluctuation of alkaline nitrogen, fat oxidation, and pH value and control the fish, so as to maintain the sensory quality of squid, prolong the freshness of squid, and improve the eating quality of squid.

Isothiocyanate is an important sulfur-containing and nitrogen-containing aromatic compound and an important intermediate in organic synthesis. Some of them were rated as safe as spices. Currently, the American Food Flavors and Extracts Manufacturers Association generally recognizes 19 isothiocyanate flavors as safe, while China GB 2760-2014 allows the use of 15 isothiocyanate flavor compounds.

Of course, isothiocyanates can also be processed into related products. The mustard bud-lyophilized powder was used as raw material, and the wet granulation process was used to affect the content of isothiocyanate granules. 75% food grade soft wood was used, and the highest content was dried at 55 °C for 60 min after isothiocyanate. The best chewable tablets were determined by orthogonal test: 30% mustard seedling powder, 20% xylitol, 0.4% citric acid, 15% maltodextrin, 0.5% magnesium stearate, and 34.1% milk powder. The quality index of chewable tablets reached the national standard, and the isothiocyanates content reached 0.25 g/100 g DW.

28.7 Patents of Isothiocyanates

Isothiocyanates are interesting and important in food; therefore innovations in processing and applying are continually emerging. We introduce here recent patents in relevant area.

28.7.1 Flavoring Agents

1. The invention relates to a vegetable seasoning powder rich in isothiocyanate and a production method thereof (Guo et al. 2016)

The vegetable flavoring powder rich in isothiocyanate contains the mature vegetable powder of cruciferous plants and its sprout powder, in which the weight ratio of the mature vegetable powder of cruciferous plants and its sprout powder is 1:1.5 and the content of isothiocyanate in the said vegetable flavoring powder is 800–2500 mg/100 g dry weight. Vegetable flavoring rich in isothiocyanate is made from cruciferous vegetables or their sprouts through selection, cleaning, heat treatment, freezing, enzymatic hydrolysis, drying, and ultra-fine grinding. Spicing with compound salt, monosodium glutamate, garlic powder, ginger powder, green onion powder, onion powder, chili powder, star anise powder, pepper powder, sterilization, packaging, prepared. The production process of the invention is simple, the formulation is reasonable and scientific, and it can be industrialized. The content of isothiocyanate in the product is 800–2500 mg/100 g dry weight.

28.7.2 Antistaling Agent

1. A method for prolonging the shelf life of meat by using aromatic extract (Fan et al. 2019)
   The method of prolonging meat shelf life with aromatic extract is simple and effective. It can safely and reliably extend the shelf life of fresh pork to 30 days and effectively achieve the preservation effect and has the advantages of green environmental protection, low power consumption, energy saving, and no secondary pollution.
   The method includes the following:

① Extract and concentrate isothiocyanate and w-hydroxyl isothiocyanate from mustard into a paste, which is used as an aromatic extract agent.
② Place the meat class of aromatic extract agent in a storage box.
③ Refrigerate box placed at 0 °C~ 8 °C freezer.

2. Mustard essential oil preservative and its application in fruit and vegetable preservation (Guan et al. 2008)
   The main active ingredient of wasabi essential oil preservative is wasabi essential oil containing more than 99% allyl isothiocyanate. The solid sustained-release tablets are composed of wasabi essential oil (5% ~ 10%), -cyclodextrin (80% ~ 85%), and stearic acid (5% ~ 10%). This antistaling agent can slowly release the mustard oil, combined with fruits and vegetables plastic film packaging, applicable to apple, pear, grape, jujube, citrus, mango, cherry, banana, potato, onion, garlic, and other fruits and vegetables preservation, suitable effective inhibition of

pathogenic microorganisms in the postharvest process of fruits and vegetables, prolong the preservation time and the distance of the market circulation.

3. The invention relates to a highly effective externally controlled preservative and a preparation method thereof (Li et al. 2007)
   The invention provides an effective externally controlled preservative, which is composed of 30% ~ 50% silica, 49.995% ~ 68% edible alcohol, and 0.005% ~ 2% allyl isothiocyanate by mass percentage. It can be widely used in the storage of food, medicine, reagents, tobacco and leather, when applied to food storage, paper film packaging bag shape and moisture permeability, small flake form placed in food packaging, make fresh drug slow release in the form of gases, evaporate into the internal space, in the fresh food forms around a certain concentration of gas phase protection layer, effectively restrain and kill several kinds of fungi and bacteria, and other corruption. At the same time, it can improve the osmotic pressure in packaging and lower food water activity, thereby effectively achieving preservation purposes.
4. Antibacterial deoxygenation dual-effect food preservative and its preparation method (Liang et al. 2008)
   The antibacterial and deoxidizing dual-effect food preservation agent is composed of a combination of the deoxidizer component and the antibacterial component, with the deoxidizer component accounting for 60–90% and the antibacterial component accounting for 10–40%. The antibacterial component includes the adsorption carrier and the antibacterial solution. The adsorption carriers include food starch, silica, vermiculite, diatomite, zeolite, bentonite, fiber, etc. The bacteriostatic solution is edible alcohol, propylene glycol or the mixture of them. Antibacterial and synergistic agents include allyl isothiocyanate, allicin, cinnamaldehyde, cinnamic acid, carvol, eugenol, thymol, and citral. It has the function of double protection of oxygen absorption and bactericidal and bacteriostatic.
5. Isothiocyanate preservative and its application (Koster et al. 2004)
   Mustard oil containing isothiocyanates has been publicly shown to have antibacterial and antifungal effects in oral therapy and in some foods. Isothiocyanate compounds in mustard oil are active agents providing antimicrobial effects. Derived from white mustard or yellow mustard, the oil also provides antibacterial and antifungal beneficial effects. However, white mustard oil is known to be a viscous oil, so it is difficult for the oil to be dispersed uniformly into solid food products or on the surface of solid food products. In addition, isothiocyanate compounds are effective antimicrobials at relatively low doses. As a result, it is difficult to distribute such a low quantity uniformly to a solid food substrate or to the surface of a solid food product. In addition, the main ingredient in white mustard oil is a moisture-sensitive compound that begins to degrade, or hydrolyze, within hours of being exposed to water. In general, the mustard processing industry mainly uses white mustard powder, while essential oils are largely ignored. In fact, in order to take advantage of white mustard powder, which has no “heat” sensitivity, the ground mustard powder has to go through a heat deactivation step. Myrosinase is deliberately catalyzed so that the essential oil

does not form when the powder is mixed with moist food products such as meat and sausage.

28.7.3 Annexing Agent

1. The invention relates to a soft yellow mustard paste which is easy to spread and a preparation method thereof (Zhang et al. 2019)
   The mustard seeds were crushed at low temperature to protect the myrosinase activity and avoid enzyme inactivation caused by high temperature, so that the glucosinolates in mustard seeds could fully generate allyl isothiocyanate, thus reducing the generation of other bitter substances. Adding appropriate amount of soybean protein can improve the stability of allyl isothiocyanate hydrolyzed from wasabi powder and make the main flavor substance isothiocyanate less volatile. The hydrolysis of allyl isothiocyanate was inhibited, and the flavor of wasabi was prolonged. The inclusion of cyclodextrin in allyl isothiocyanate makes the product taste mellow and soft, without pungent irritation, and increases the acceptability of the product. Intermittent ultrasonic emulsification homogenization makes mustard sauce more uniform and better applicability, and flexible sterilization is adopted in the later stage to better retain the flavor of the product. The products are prepared by activation, preliminary crushing, ultra-fine crushing, heating and dissolution, stirring, hair preparation, ingredients, phacoemulsification, and sterilization.
2. Complexes for immobilizing isothiocyanate natural precursors in cyclodextrins, preparation, and use (Roselli et al. 2004)
   The product of isothiocyanate embedded in cyclodextrin has antibacterial and bactericidal properties. The organic isothiocyanates are stable as inclusion compounds in cyclodextrin, allowing them to dissolve in aqueous media and controlling their release. The antimicrobial, bactericidal, and/or fungicidal composition of this compound can be used in the food and food processing fields. Their physical properties make them simple for use in food and food processing applications, and thus the dosage of the preservation process can be controlled without compromising the sensory properties of the food to be preserved.

28.7.4 Other Patents

1. Application of isothiocyanates in the preparation of herbicides for the control of weeds in fields and lawns (Lin et al. 2010)
   The application of a group of isothiocyanate compounds in the preparation of agricultural herbicides for the control of weeds in farmland and lawn is provided, which can be used as single agent or compound agent and can be made into soluble liquid agent, water emulsion, oil agent, emulsion, or other dosage forms.
2. Application of isothiocyanates in promoting hair growth (Cheng et al. 2009)

Natural and synthetic isothiocyanate compounds or their derivatives and metabolites have broad clinical application and market development prospect in promoting hair growth, especially in the treatment and prevention of hair loss.

28.8 Perspectives

The isothiocyanate is mainly hydrolyzed by glucosinolates in the family Brassicaceae and Brassica. There are many types; common are benzyl isothiocyanate, allyl isothiocyanate, indole-3-carbinol, phenethyl isothiocyanate, sulforaphane, sulforaphene, iberin, and erucin. Their structures are different and their functions are different in all aspects. The absorption of isothiocyanates in the human body also depends on the degree of hydrolysis of the glucosinolates. The type of vegetables, the chewing of the mouth, the pH of the environment, and the action of microorganisms all affect the hydrolysis of glucosinolates. The bioavailability of isothiocyanates can be understood by measuring metabolites in blood and urine. These are the basis for studying the mechanisms of their human protection effects.

Isothiocyanates possess a lot of biological activity, which can effectively inhibit cancer tumors and treat diseases and antibacterial. At present, the research mainly focuses on anticancer, and it can exert its activity by inducing various signal transductions such as apoptosis, thereby enhancing the human immune system. Consumption of isothiocyanate has great benefits for human health, and it can also play a good role in food preservation. Whose activities of antibacterial and antiviral have a good application in controlling food diseases, which is also an important direction for the development of isothiocyanates in the food industry in the future.

With the development of extraction and analysis technology, more and more isothiocyanate compounds will be identified from food, and their application in the food field and even in the medical field will be expanded. Isothiocyanates currently used in the food industry are mainly concentrated food flavors compounds. The unique spicy pungent odors of isothiocyanates make mustard oil, mustard sauce, kimchi, sauce, and other foods and condiments be more attractive and popular. Secondly, the antibacterial properties of isothiocyanates also make them apply to food preservation. At the same time, some physiological activities of isothiocyanates such as antitumor and anti-oxidation can be used as components of functional foods and can positively promote people's health. However, isothiocyanates are affected by the cooking method and time during cooking. High temperature and long-term heating can accelerate the destruction of isothiocyanates. Thus, cooking should be carried out in the shortest possible time to minimize the loss of isothiocyanate.

Although isothiocyanates have a variety of physiological functions and are beneficial to a human body within a certain range, pure isothiocyanates have acute toxicity, chronic toxicity, and genotoxicity. Excessive intake of isothiocyanate can cause poisoning in humans or animals. Under normal circumstances, the daily intake of isothiocyanates from cruciferous vegetables such as broccoli and broccoli is not enough to cause high doses of poisoning. Although there is a certain amount of isothiocyanates in the seasonings such as mustard sauce, mustard oil, and kimchi, the

seasoning is originally used as an auxiliary seasoning for food, and the daily intake is small. It's not easy to cause food poisoning.

In general, this chapter provides an overview of the classification, bioavailability, metabolism, functional activity, application in food, and patented products of isothiocyanates. What is the next major development in the study of isothiocyanates?

Many extrinsic and intrinsic factors can influence the final uptake, formation, bioaccessibility and bioavailability of isothiocyanate. A great deal of efforts has been made to grow Brassica vegetables containing much greater levels of glucosinolates contents in order to understand the effects of industrial processing on the glucosinolate-myrosinase system, to optimize these processes, to indicate the bioaccessibility and bioavailability of isothiocyanate, and to study the beneficial therapeutic effects of isothiocyanates in vitro and in vivo. However, still many factors and mechanisms need to be researched in further study. Factors that affect the bioaccessibility/bioavailability of glucosinolates and isothiocyanates such as meal composition are not yet clear, and more studies should be performed to elucidate how the final isothiocyanate absorption can be enhanced during consumption of a meal.

People pursue a higher quality of life including food and clothing. In order to improve food quality, researchers have been trying to constantly promote the development of science and technology. Isothiocyanates have been shown to reduce the risk of cancer; therefore they will be studied intensively. Isothiocyanate has the potential to prevent the complexities associated with cancer. Cruciferous vegetables are considered to be one of the significant sources of glucosinolates availability required for isothiocyanate formation. Recently the public and scientific community focuses on the beneficial properties of isothiocyanate. This has caused a demand of functional foods and dietary supplements rich in isothiocyanate concentrations in the United States and Europe health food markets. The health effects of isothiocyanates are promising, but there are specific limitations and challenges. Further research is needed to determine in depth the biological activities of various glucosinolates in order to determine their advantages and disadvantages.

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Polysaccharides in Food

29

Junqiao Wang, Junyi Yin, and Shao-Ping Nie

Contents

Abstract

The polysaccharide is widely found in various food sources, including mushrooms, seaweeds, cereal grains, etc. The structure of polysaccharide is usually complicated, so a combination of chemical methods, instrumental technologies, and biological methods are always applied simultaneously in order to fully understand the primary structure. It is widely accepted that they have many bioactivities, such as immunomodulatory activity, anticancer activity, antioxidant activity, antidiabetic activity, renal protective and liver protective function, and so on. In this chapter, an overview of the latest developments in isolation,

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purification, structural properties, and bioactivities of food polysaccharide is discussed and summarized.

Keywords

Polysaccharide · Isolation · Purification · Structure · Bioactivities

29.1 The Definition and Research History of Polysaccharides

Polysaccharides are widely distributed in food resources like plants, animals, and mushrooms, and they are one kind of major and essential bio-macromolecules in living organisms together with proteins and polynucleotides. The monomeric units, known as monosaccharide unit, of polysaccharide are mutually jointed by various types of linkages. Polysaccharides are condensation polymers where the glycosidic linkage formed from the glycosyl moiety and a hydroxyl group of the adjacent sugar unit. Most of them exhibited as linear or branched chains with exception of cyclic polysaccharides named cycloamyloses. Based on the number of monosaccharide presented, polysaccharides can be divided into two class, homopolysaccharides and heteropolysaccharides. Researches on the chemistry of simple sugars began in the late nineteenth century by Fischer (1893). The ring structure was determined in 1920–1940 by Haworth and his colleagues (Haworth 1925, 1928; Haworth and Hirst 1930). Since then, more and more scientists were interested in the studies of sugar chemistry. Currently, starch, cellulose, chitin, and other polysaccharides have been engaged in extensive researches and characterized by classical chemical methods. In 1988, Dr. Dwek from University of Oxford published a review titled "Glycobiology" (Rademacher et al. 1988), which was very important in the sugar research history. In recent years, the study of polysaccharide emphasized on their utilization in the intestine and the beneficial impact on colon health. More and more reports in Nature and Science announced the functionality of polysaccharides. Furthermore, due to the rapid development of technologies, it is more accurate and convenient to characterize the chemical structure of polysaccharides. The extensive use of NMR and MS technologies made it possible to analyze polysaccharides with complex structure properties.

29.2 Isolation and Purification of Polysaccharides

Polysaccharide usually exists in the cell wall of food sources. Hot water extraction is the most popular approach for polysaccharide due to a safety and environment friendly compatibility. In order to accelerate the dissolution of polysaccharide from cell walls, high-temperature, ultrasonic, microwave, enzyme hydrolysis is frequently utilized. Acidic and diluted alkali solutions are also commonly used to extract

polysaccharides in the basic rule of breaking cell walls from outer to inner layer. Generally, the traditional procedures for polysaccharide extraction are conducted as follows:

1. Firstly, powder of food materials are defatted by organic solvents in order to eliminate part of low molecular substance, such as pigments and lipids that may be co-extracted by water.
2. Then, the dried defatted materials are extracted using hot water, acidic solution, and diluted alkali solution. Furthermore, some assistant methods are used to improve the extraction process.
3. Afterward, the extract solutions are deproteinized by Sevag method to remove the free protein, and in some cases, protease or proteinase would be added to polysaccharide solutions to remove both the free and covalently linked proteins. After dialyzing against water to remove small molecular compounds, the deproteinized solution is precipitated by alcohol to obtain a crude polysaccharide.

Purification process is conducted for preparing pure polysaccharides using a combination of techniques, such as fractionation by ethanol precipitation, ultrafiltration, freeze-thawing method, and different column chromatography methods. The most common chromatography methods are ion-exchange chromatography and gel filtration chromatography, based on the separation principle of ion strength properties and molecular size differences, respectively.

29.3 Chemical Structure of Bioactive Polysaccharides

The structure of polysaccharide is usually complicated, and determination of its structure requires a combination of chemical methods and instrumental technologies, in addition to biological method. To understand the primary structure, the information of monosaccharide composition, molecular weight, glycosidic linkages and sequences, ring size, anomeric configuration, and substitution (if appropriate) is essential. The polysaccharides extracted from food materials are generally purified prior to structural characterization. Therefore, the first step for analyzing a polysaccharide is determination of its molecular weight distribution and purity. And the second step is identification of monosaccharide composition that reflected the availability of purification procedure and unveiling structural information to a certain extent. The detailed principles, advantages and disadvantages, and precautions of methods for polysaccharide characterization have been comprehensively described in several reviews (Nie et al. 2018b; Xie and Nie 2010; Xie et al. 2017; Yang and Zhang 2009; Yin et al. 2018). Especially, a comprehensive utilization of "partial degradation-methylation-NMR" strategy was put forward by Yin, Xie, and Nie, for characterizing the fine structure of polysaccharides with complex structure (Yin et al. 2018).

29.3.1 Bioactive Polysaccharide from Edible Mushrooms

29.3.1.1 Glucan

Mushroom is a kind of fungi that is popular for its special flavor and nutrition. In many Asian countries, like China, Japan, and Korea, mushrooms such as Lingzhi (*Ganoderma lucidum*), maitake (*Grifola frondosa*), and Shiitake (*Lentinus edodes*) have been harvested, cultivated, and consumed for hundreds of years, being valued as edible/medicinal sources. These mushrooms represent an unlimited resource of polysaccharides with a distinctive antitumor and immunomodulating activity. Both naturally collected and artificially cultivated fruiting bodies of mushrooms are contained bioactive polysaccharides. The fungal walls are mainly composed of structural polysaccharide. Polysaccharides extracted from mushroom are regarded as one of the primary bioactive constituents and are beneficial to human health. The most common polysaccharides contained in edible mushroom are α-, β-, or mixed glucans. Among them, β-D-glucan with a (1→3)-linked main chain substituted at O-6 is a typical structure observed in various mushrooms, such as lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, grifolan from *Grifola frondosa*, and scleroglucan from *Sclerotium sclerotia*. Besides, Linear (1→3)-β-D-glucan or (1→3)-β-D-glucan with some different branch chains, such as (1→2)-, (1→3)-, (1→4)-, and (1→6)-linked Glc*p*, was commonly observed in mushrooms. Such kind of chemical structures has been proved to be responsible for some biological properties, for example, antitumor, immunomodulatory, and antioxidant activities. A glycogen-like structure, α-D-glucan with main chain of (1→4)-linked Glc*p*, was also found in some mushroom polysaccharides, such as *Agaricus bisporus* (Smiderle et al. 2010), *Coprinus comatus* (Li et al. 2013), and *Cordyceps sinensis* (Wang et al. 2017b, c). Additionally, polysaccharide with (1→6)-β-D-Glc*p* backbone was reported in mushrooms of *Calocybe indica* (Mandal et al. 2010), *Entoloma lividoalbum* (Maity et al. 2014), *Pleurotus citrinopileatus* (Liu et al. 2012), and *Russula albonigra* (Nandi et al. 2014), of which mostly by alkali extraction. The differences observed among these D-glucans are the types and degree of branching, which usually depends on the sources, extraction solvent, or isolation procedures.

29.3.1.2 Heteromannan

Heteromannans are another kind of polysaccharides found in mushrooms, especially in ascomycetes (Leal et al. 2010; Ruthes et al. 2016). They consist part of the cell wall structure of mushrooms and can also be attached to proteins forming the cell wall matrix (Ruthes et al. 2016). Generally, the heteromannans are extracted by diluted alkali solution (NaOH or KOH). Their solubility in water enables separation from other water-insoluble polysaccharides through dialysis, and the solubility in 50% ethanol improves the purity. The heteromannan displayed fairly similar structure, always an α-(1→6)-linked mannan core constitutes the main chain, more or less substituted by α-Man*p* and/or β-Gal*f* containing side chains. The molecular weight of these polysaccharides ranged from 15 to 100 kDa (Leal et al. 2010).

29.3.1.3 Heterogalactan

In addition to glucan and heteromannan, heterogalactans have been characterized in mushroom, especially in *Basidiomycetes*. In most cases, this polysaccharide showed a (1→6)-α-Gal*p* backbone with O-2 substitution and can also be partially *O*-methylated with 3-*O*-Me-α-D-Gal*p* residues presented at the main chain (Ruthes et al. 2016).

29.3.2 Functional Polysaccharide from Seaweed

Marine macroalgae (seaweeds) are commonly consumed in daily diet, especially in Asian. They can be classified into brown algae (phaeophyta), green algae (chlorophyta), and red algae (rhodophyta) based on the chemical composition (Gupta and Abu-Ghannam 2011). Seaweeds contain unique, hydrocolloid polysaccharides such as alginate and carrageen that have been applied in many food industries. Importantly, they are also rich sources of naturally sulfated polysaccharides, which possessed a number of biological activities, such as antiviral, anticoagulant, immunomodulatory, etc. (Wijesekara et al. 2011; Wijesinghe and Jeon 2012). The major sulfate polysaccharides identified in marine algae were fucoidan and laminarans from brown algae, ulvan from green algae, as well as carrageenan from red algae.

29.3.2.1 Fucoidan

Fucoidan is a type of natural sulfated polysaccharide mainly distributed in the cell wall matrix of brown seaweeds, like *Fucales*, *Laminariales*, *Chordariales*, *Dictyotales*, *Dictyosiphonales*, *Ectocarpales*, and *Scytosiphonales* (Gupta and Abu-Ghannam 2011). It is a branched polymer containing substantial amount of L-fucose and sulfate ester groups. Actually, this kind of sulfated polysaccharide has been characteristically identified in all brown algae so far and seems not in green algae, red algae, as well as in freshwater algae. The structure of fucoidan differs among seaweed species and was predominantly composed of (1→3)-α-L-fucose main chain or alternating (1→3)-α-L-fucose and (1→4)-α-L-fucose, (1→2)-α-L-fucose sometimes presented in the backbone. Sulfate groups usually occupied in C-2 and/or C-3 and C-4 position of fucose. Specifically, it is worth noting that fucoidan is not a pure fucan sulfate but a heteropolymer containing fucose, galactose, xylose, mannose, or glucuronic acid.

29.3.2.2 Laminarin

Laminarin, found in *Laminaria* and *Saccharina* species, is low molecular weight polysaccharide and bioactive constituent in brown algae. As a food reserve in brown algae, the content of laminarin is up to 35% of dry weight (Kadam et al. 2015). It contains 20–25 glucose monomers constituted (1→3)-β-D-Glc*p* main chain and certain (1→6)-β-D-Glc*p* branching. There are two types of laminarin chains differ in the reducing end. One is end with a mannitol residue, and the other is end with

a glucose residue. The molecular weight of laminarin is approximately 5 kDa dependent on the degree of polymerization.

29.3.2.3 Ulvan

Ulvan is the major water-soluble polymer found in green seaweed of two major genera, Ulva and *Enteromorpha* sp., accounting for around 8–29% of the dry weight (Lahaye and Robic 2007). The extraction is generally conducted by water solutions containing a divalent cation chelator at about 80–90 °C and accumulated by alcohol or quaternary ammonium salt precipitation (Lahaye and Robic 2007). As reviewed by Lahaye and Robic (2007), ulvan structure shows great complexity and variability, and determination of the sugar sequence is a major challenge. Sulfate, rhamnose, xylose, iduronic, and glucuronic acids are the main components of Ulvan. Specifically, the major repeating disaccharide unit is ulvanobiouronic acid 3-sulfate type containing either glucuronic acid or iduronic acid (Jiao and Yu 2011).

29.3.2.4 Carrageenans and Agarans

For red seaweed, the sulfated polysaccharides are primary galactans called agarans and carrageenans. The two galactans differs in stereochemistry, of which with 4-linked α-galactose of L-series termed as agarans and those of D-series termed as carrageneens. Especially, carrageneens are widely used as food additives at present. The repeating disaccharide units of carrageneens are composed of alternating 3-linked β-D-Gal*p* and 4-linked α-Gal*p* or 3,6- anhydro-α-Gal*p*, as showed in Fig. 1.

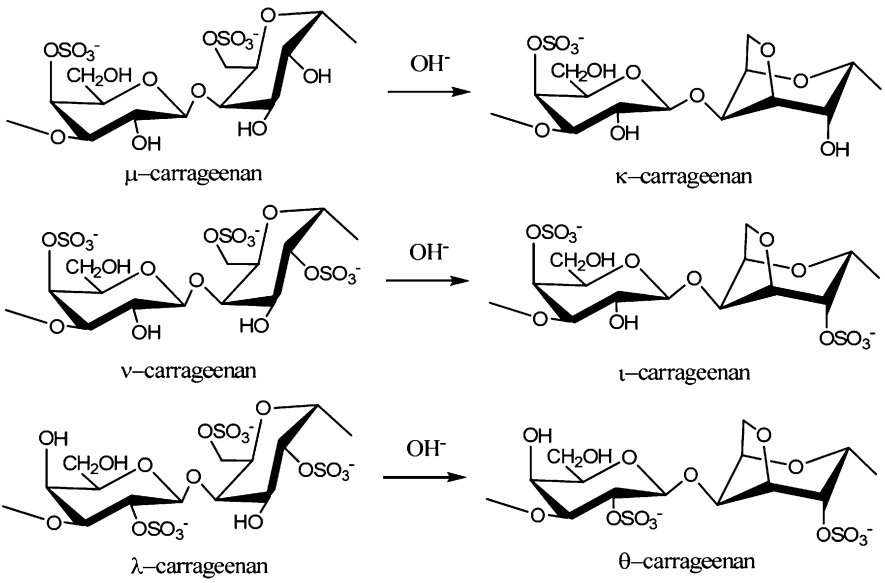

Fig. 1 The repeating disaccharide units of different types of carrageenan and their transformation by treatment with alkali (Jiao and Yu 2011), https://doi.org/10.3390/md9020196

29.3.3 Bioactive Polysaccharide from Cereal Grains

Cereal grains, such as rice, wheat, barley, oat, rye, maize, and sorghum, are staple foods for the majority of population all around world, especially for the Western diets. The bioactive polysaccharides in cereals, mainly referring to the non-starch polysaccharide derived from cell walls, account for approximately 3–8% of the grain (Bader Ul Ain et al. 2018), and it considered as an excellent source of soluble and insoluble dietary fiber.

29.3.3.1 Arabinoxylan and (1→3) (1→4)-β-Glucan

The noncellulosic polysaccharides presented in cereals are predominantly arabinoxylans, β-glucans, pectins, and arabinogalactans. Among them, arabinoxylans and mix linked (1→3) (1→4)-β-glucans are predominant cell wall structural components and bioactive polysaccharides. The cell walls of barley and oats contained generally higher levels of β-glucan, whereas rye and wheat cell walls were abundant in arabinoxylans (Bader Ul Ain et al. 2018; Cui and Wang 2009; Muralikrishna and Subba 2007). Both of them have been characterized extensively due to their promising functional properties. The structure of cereal β-glucan is a typical linear homopolysaccharide, and over 90% of them was made up of two or three consecutive (1→4)-linked Glc*p* separated by a single (1→3)-linked Glc*p*, forming as two building blocks, a trisaccharide or tetrasaccharide unit. For arabinoxylans, it is composed of a linear xylan backbone with β-(1→4)-linkage, a ferulic acid moiety, and an α-L-Ara*f* substitution along the backbone. The Ara*f* residues are attached to the backbone at C-3 and/or C-2 position. While the majority of Ara*f* substitutions are monomeric, a small proportion of Ara*f* were found as short oligosaccharide side chains with (1→2)-, (1→3)-, and (1→5)-linked Ara*f* residues. In addition, xylose and galactose are reported existed in the reducing end in some cases. Uronic acid, galactose, glucose, and acetyl groups were substituted at O-2 and/or O-3 of the xylan chain in much lower amount (Shuangyue et al. 2015). This suggested that the general structural characteristic of arabinoxylan varies significantly among cereal sources and cultivars.

29.3.3.2 Fructans

Fructans, also called polyfructosyl sucroses, consist mainly or exclusively of fructose and contain no or one glucose (Lewis 1993). They have many different types of structures, ranging from three up to a few hundred monomer unit and could be classified based on the types of fructose-fructosyl linkages, β-(2→1)-D-Fru*f* or β-(2→6)-D-Fru*f*, as well as their core molecule. Inulin was the common known inulin-type fructans, which had a linear chain of almost exclusively β-(2→1)-D-Fru*f* residues. Fructans of levan-type were mainly consisted of linear β-(2→6)-D-Fru*f* units. Gramminan-type fructan was built up of both β-(2→1)-D-Fru*f* and β-(2→6)-D-Fru*f* linkage. Neo-inulin and neo-levan-type fructans were composed of 6G-kestotriose and have predominantly β-(2→1)-D-Fru*f* and β-(2→6)-D-Fru*f*, respectively. The structure of these five fructan types could be clearly overviewed by Fig 2. As reviewed by Verspreet et al., rye contained the highest levels of fructan among

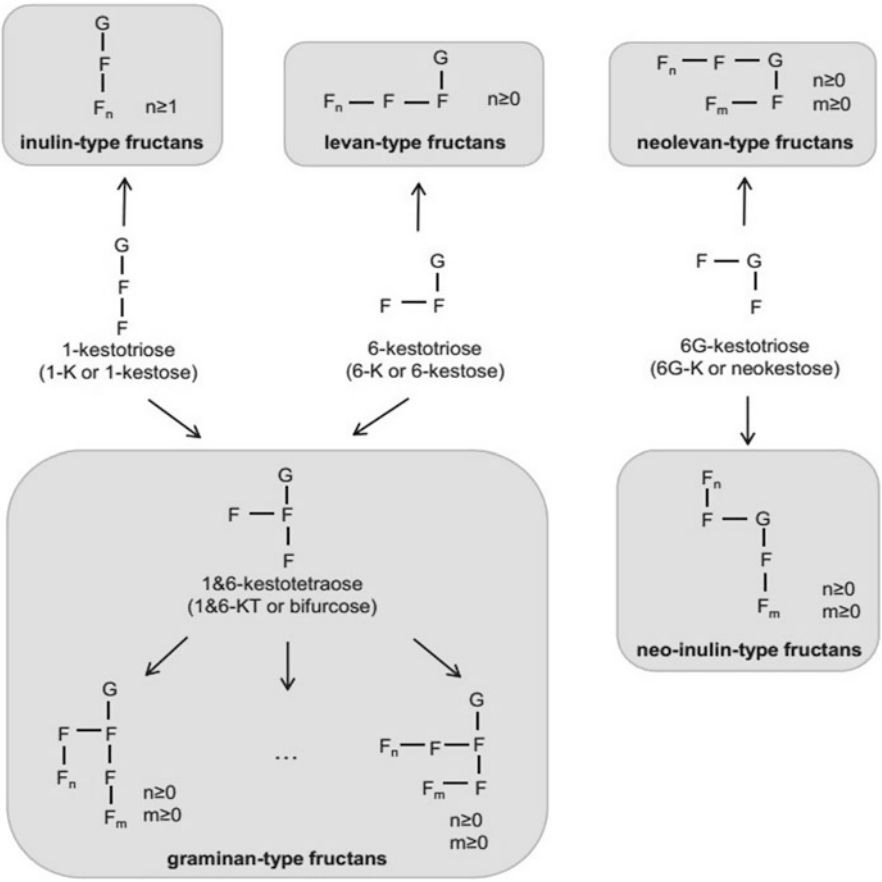

Fig. 2 Overview of the five different fructan types. G and F represent glucose a fructose unit, respectively. Vertical and horizontal lines between two fructose units depict β(2,1)-linkages and β(2,6)-linkages, respectively (Verspreet et al. 2015), reprinted with permission from Elsevier

cereal grains, of which concentrations ranged from 3.6% to 6.6% of dry weight (Verspreet et al. 2015). Cereal grain fructans comprise a complex mixture of mostly branched structures. These fructans had a profound positive effect on colon health.

29.3.4 Bioactive Polysaccharide from Vegetables and Fruits

Food polysaccharides are proved to have certain beneficial effects on the diet and human health, such as immunomodulatory, antitumor, anti-inflammatory, antibacterial, hypoglycemic, and anticancer activities. Fruits and vegetables play a crucial part in human diet and daily life. They are abundant in soluble and insoluble dietary fiber, such as hemicelluloses and pectins. Traditionally, dietary fiber was

defined as "plant polysaccharides (including cellulose, hemicelluloses, gums, oligosaccharides, and pectin) and lignin which are resistant to hydrolysis by digestive enzymes" by AOAC International and AACC International in the 1970s (Prosky et al. 1985). Recently, a more comprehensive description of dietary fiber by Codex was defined as "carbohydrate polymers with ≥10 (or 3, depending on the jurisdiction) monomeric units that are resistant to enzymatic hydrolysis during human small intestinal digestion" (Codex 2015).

On the other hand, fructans, another kind of dietary fiber, were also naturally presented in some vegetables and flowers, like taproot of chicory, tubers of dahlia, bulbs of tulip, and onion (Ritsema and Smeekens 2003). Fructans are also a kind of reserve carbohydrate in some edible plants. Due to the similarity structures of fructans in cereal grains, it will not list separately in this part.

29.3.4.1 Pectin

Plant acidic polysaccharides, particularly pectic polysaccharides, are widely distributed in the primary cell walls of roots, stems, leaves, fruits, etc. of all land plants. Pectic polysaccharides have a complex structure composed of up to 17 monosaccharides with more than 20 different linkages (Ndeh et al. 2017; Voragen et al. 2009). But to some extent, it shares common features, particularly the presence of galacturonic acid in the backbone giving these polysaccharides anionic properties. Besides, it also comprises a few neutral polysaccharide chains such as arabinans and arabinogalactans that always interconnecting the anionic moieties. Generally, the major pectic polysaccharides are recognized as homogalacturonan (HG), rhamnogalacturonan-I (RG-1), and rhamnogalacturonan-II (RG-II). HG was composed of linear α-D-(1→4)-Gal*A* residues with some of C-6 carboxyl groups methyl-esterified, while RG-I was made up of alternating disaccharide repeating unit of →4)-α-D-Gal*A*-(1→2)-α-L-Rha(1→ with various glycan chains attaching to the backbone (mainly arabinose and galactose) (Koubala et al. 2014; Mohnen 2008; Willats et al. 2006). RG-II, a relative minor component of cell wall, is considered to be the most structurally complex polysaccharide with about 12 different monosaccharide residues interconnected by more than 20 types of glycosidic linkages. It consisted of a homogalacturonan backbone comprising seven to nine →4)-α-D-Gal*A*-(1→ residues, and some of them was methyl-esterified in addition to a highly branched side chains which vary with the source of fruits and vegetables. Different pectin fractions were extracted from cell wall materials of fruits and vegetables using water, sodium carbonate (Na_2CO_3), and chelate agent like 1,2-cyclohexanediaminetetra-acetic acid (CDTA). Specifically, CDTA and Na_2CO_3 were employed to extract pectin that was ionically or covalently bound to the cell wall materials (Lara et al. 2004; Posé et al. 2012; Redgwell et al. 1992; Selvendran 1985). CDTA-mediated extraction released pectin from the cell wall by complexing calcium, which was mainly distributed in the middle lamella containing a relatively high content of galacturonic acid. Na_2CO_3 extraction was able to release the neutral sugar-rich pectin from the primary cell wall by breaking ester linkages.

29.3.4.2 Xyloglucan

Xyloglucan is a matrix polysaccharide that is also presented in the cell walls of most land plants, and the best characterized xyloglucans are from tamarind seeds (Popper et al. 2011). Xyloglucan possessed a linear backbone of (1→4)-β-D-glucan, in this sense identical with cellulose. The backbone is composed of a cellotetraose with the first three glucose, counting from the nonreducing end, carrying an α-D-xylose residue on position 6 and the fourth glucose residue unsubstituted. Some of the xylose residues carry additional sugars, especially D-galactose and L-fucose.

29.3.4.3 Glucomannan

Glucomannan is widely distributed in conifers, the corms of *Amorphophallus* konjac and dicotyledons like *Dendrobium*, Aloe, in addition to the microorganisms or fungal cell walls. They are neutral polysaccharides acting as a source of soluble dietary fiber with a particular feature of high viscosity. They are constructed from mannose as the primary residues and galactose containing side chains. In most cases, these polysaccharides present a β-(1→4)-linked backbone containing Man*p* and Glc*p* residues, with some acetyl substituent. For example, konjac glucomannan is composed of β-D-(1→4)-Man*p* and β-D-(1→4)-Glc*p* with reported ratio of 1.6:1 or 1.4:1(Zhang et al. 2014). There are acetyl groups attaching randomly to C-6 position of approximately 1 per 19 sugar residues and some side chains linking to Man*p* residues by joint C-3. On the other hand, *O*-acetylated glucomannan isolated from *Dendrobium huoshanense* and *Dendrobium officinal* was found to substitute at O-2 and O-3 position of β-D-Man*p*, as shown in Fig. 3 (Xing et al. 2014, 2015). Polysaccharides from leaf skin of aloe vera were identified as *O*-acetyl-β-(1→4)-glucomannans, with the acetyl groups attaching to O-2, O-3, and/or O-6 positions (Shi et al. 2018a, b). The degree of acetylation and the ratio of mannose and glucose depend on the raw materials, affecting the properties of the polymer.

29.3.4.4 Galactomannan

As the name suggested, the polysaccharide is composed of two kinds of monosaccharides with mannose as the major component and galactose as the minor one. Unlike galactomannans found in the mushrooms, plant galactomannans are water-soluble β-(1→4)-linked D-mannan with side chains of single α-(1→6)-linked D-Gal*p* residue. The number of galactose units in plant galactomannans is commonly less than that the mannose units. They are mainly found in tara, guar, locust bean or carob seeds, etc. This kind of polysaccharide is functionalized as storage non-starch carbohydrate reserved in endosperm walls and vacuoles of seeds and vacuoles in

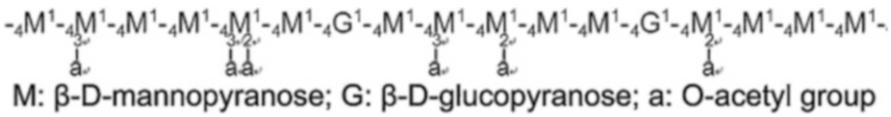

Fig. 3 Chemical structure of Dendronan from *Dendrobium officinale* (Xing et al. 2015), reprinted with permission from Elsevier

vegetative tissues (Meier and Reid 1982). The promising rheological properties of the plant galactomannans, such as thickening and additive effects, made them employ in diverse industrial and food applications.

29.4 Bioactivities (Animal Aspects)

It is widely accepted that naturally derived polysaccharides have many bioactivities, such as antitumor activity, immunomodulatory activity, gastrointestinal benefit activity, liver protection activity, hypoglycemic function, hypolipidemic function, antivirus activity, and so on. The unique structural features and biological properties of polysaccharides can be utilized successfully in various pharmacological, nutraceutical, and functional applications. Therefore, some of the bioactive polysaccharides have been made into clinical drugs or medicines for the treatment of certain diseases. One advantage for the clinical application of polysaccharides is that these carbohydrates are noncytotoxic to body cells.

29.4.1 Immunomodulatory Effect

The bioactive polysaccharides could interact with the immune systems directly or indirectly, triggering diverse cellular and molecular events and leading to the activation of immune function. The reported immunostimulatory polysaccharide included glucans, mannans, pectics, galactans, fucoidans, arabinoxylans, arabinogalactan, and other bioactive polysaccharides from plants, edible fungi, and algae. The β-(1→3)-glucans with triple helical conformation regarded as crucial structural characteristics for immunostimulating activity. For example, (1→3)-β-D-glucans that had β-D-Glc*p* units attaching by (1→6) linkages as single unit branches systemically enhanced the immune system (Bohn and BeMiller 1995). A highly branched β-glucan from the spores of *Ganoderma lucidum* significantly promoted dinitrochlorobenzene-induced delayed-type ear swelling in mice (Wang et al. 2017d). The backbone of immunostimulatory mannans mainly consists of (1→4)-D-Man*p*, especially from aloe vera, showing immuno-pharmacological and therapeutic properties. Huang et al. pointed out that β-(1→4)-D-Man*p* and *O*-acetyl structure might be responsible for the immunomodulatory activity of polysaccharide from *Dendrobium officinale* (Huang et al. 2015).

The immunomodulatory effect of food polysaccharides on cyclophosphamide (Cy or CTX) induced immunosuppressed mice was extensively studied. Food polysaccharides were intraperitoneally (in some cases) or orally administrated (in most cases) to mice after intraperitoneally injection of Cy to induce a chemotherapeutic injury. Recoveries of spleen and thymus indexes, activation of macrophage phagocytosis and NK cells, enhancement of splenocyte proliferation (cell-mediated immunity), in addition to an increased cytokine production, and serum hemolysin (humoral immunity) were observed after administrated with polysaccharides (Chen et al. 2016; Fei et al. 2016; Gong et al. 2015; Huang et al. 2015; Liu et al. 2017a;

Yang et al. 2017; Yuan-Yuan et al. 2014; Zhang et al. 2017c; Zheng et al. 2017; Zou et al. 2018). The immunoglobulin levels, like immunoglobulin (Ig) G, IgM, and IgA, in mice serum were also significantly increased (Hao and Zhao 2016; Zheng et al. 2017). Polysaccharides were also reported to ameliorate CTX-induced immunosuppression through reducing the apoptosis and oxidative damage (Li et al. 2017c). The intake of litchi pulp polysaccharides improved intestinal mucosal immune function via stimulating mesenteric lymph node cells proliferation and secretion of serum IgA (Fei et al. 2016). A macromolecular polysaccharide from *Grifola frondosa* could effectively reverse the downregulation of mRNA levels of cytokines of splenocyte in mice, in addition to increase the levels of lactate dehydrogenase (LDH) and acid phosphatase (ACP) in the spleen, as well as IL-2, IL-6, IL-1β, and IFN-γ mRNA expressions in splenocyte (Xiao-Lei et al. 2015).

Food polysaccharides might activate the intracellular signaling cascades in immune systems mediated primarily by specific pattern recognition receptors (PRRs) in the cell membrane, such as scavenger receptors (SRs) and toll-like receptors (TLRs). Activated PRRs could further enhance adaptive immune responses through the promotion cytokine productions that strengthened the cellular and humoral immune responses. Zhou et al. demonstrated that *Astragalus* polysaccharides might modulate immunity through activation of TLR-mediated MyD88-dependent signaling pathway (Zhou et al. 2017).

The improvement of immune dysfunction by polysaccharides was demonstrated to be mediated by intestinal mucosal immunity. Sheng et al. found that polysaccharide from *Hericium erinaceus* upregulated the production of secretory immunoglobulin A (sIgA) and activated the MAPK and AKT cellular signaling pathways in the intestine (Sheng et al. 2017). Yu et al. also demonstrated *Ganoderma atrum* polysaccharide (PSG-1) increased levels of phosphorylation of MAPKs and Akt, in addition to NF-κB expression in peritoneal macrophage. Ca^{2+} concentration and PKC activity of spleen lymphocytes, as well as cAMP level and PKA activity in PSG-1 groups, dramatically increased as compared with that of the model group (Yu et al. 2015). As was known, it was difficult for polysaccharides to be digested and absorbed in the intestine directly. Therefore, in recent years, many studies preferred to investigate the impact of polysaccharides on intestinal microbiota and interaction between polysaccharide-induced microbiome alteration and host immune system using approaches like multi-omics. Polysaccharide showed a prominently improvement in the intestinal immunity indexes in mice. Tang et al. found that polysaccharides from purple sweet potatoes modulated the intestinal bacteria of increasing SCFA-producing *Lachnospiraceae* and *Oscillospira* and reducing *Alcaligenaceae* and *Sutterella* (Tang et al. 2018). Zhang et al. determined a dynamic profile of mouse gut microbiome response to the intake of longan polysaccharide and thus constructed a correlation network by polysaccharide, bacterial species, microbial metabolic pathways, SCFAs, and the immune index that was better illustrate the causality (Zhang et al. 2017a). The protective effect of fungal polysaccharides from *Cordyceps sinensis* (CSP) and *Ganoderma atrum* (PSG), as well as their combinations on colon immune dysfunction induced by Cy, was studied, respectively, by Fan et al. Result showed that CSP significantly promoted microbial-derived butyrate to

improve histone h3 acetylation mediating regulatory T (Treg)-cell-specific Foxp3, as well as significantly restored Cy-induced elevation of IL-17 and IL-21. On the other hand, PSG significantly downregulated MyD88, in addition to increased IL-10 and TGF-β3. The combination of two polysaccharides balanced the disequilibrium of cytokines secretion and Foxp3/RORγt ratio-related Treg/T-helper 17 (Th17) balance, as well as downregulated the TLR-mediated inflammatory signaling pathway and promoted secretory immunoglobulin A (sIgA) secretion to suppress colonic inflammation (Fan et al. 2018). In summary, it is believed that polysaccharide-derived alterations in intestinal microbiota and microbial-associated inflammatory signals might be involved in the polysaccharide immunomodulatory activities.

29.4.2 Anticancer Effect

Polysaccharides from plants and mushrooms have potent anticancer activities (Zong et al. 2012). Unlike the tradition anticancer chemotherapy drugs, polysaccharides are nontoxic with small or no side effects. They provided therapeutic benefit in tumor-bearing mice. The antitumor polysaccharide varied from glucan, mannan, and hemicelluloses to heteroglycan. Among them, mushroom polysaccharides have been used in clinical anticancer therapy in some countries, particularly β-D-glucans (Zhang et al. 2007). In 1967, Chihara et al. reported that the polysaccharides from *Lentinus edodes*, lentinan and the β-(1→4),(1→6) linked glucan, showed a prominent inhibitory effect on the tumors growth in Sarcoma 180-bearing mice (Chihara et al. 1969, 1970). Since then, the antitumor activity of polysaccharide has aroused attention worldwide, much focusing on discovering novel antitumor adjuvant resources. Particularly, it has been proved that polysaccharides with structural characteristics of β-(1→3)-linkage in the main chain of glucan with β-(1→6)-branching points were effective in antitumor ability (Wasser 2002). In addition, β-(1→3)-glucans with triple helical conformation are regarded as a key structural feature of bioactivities. The tertiary structure of lentinan was lost when denatured with DMSO, urea, or sodium hydroxide, without changes of its primary structure. However, it was interesting to observe a lowered tumor inhibitory ability by progressive denaturation treatment (Maeda et al. 1988).

In addition to the well-known antitumor β-(1→3)-glucans, a wide range of biological glucans with other structures have been described. Masuda et al. revealed that maitake α-glucan, a highly branched α-(1→6)-branched α-(1→4)-glucan, significantly inhibited tumor growth and improved survival rate of colon-26 carcinoma- and B16 melanoma-bearing mice. The oral administration of this polysaccharide directly activated macrophages and dendritic cells in Peyer's patches and induced Th1/CTL immune response so as to inhibit the tumor growth (Masuda et al. 2017). Polysaccharide from fruit bodies of *Auricularia auricula-judae* (AAG) with a main chain of β-(1→4)-glucan and side chains of β-(1→6)-D-Glc*p* showed a significant inhibitory effect on tumor growth in a dose-dependent manner with no cytotoxicity. Furthermore, AAG-induced apoptosis in S-180 tumor cells by upregulating Bax and downregulating Bcl-2 (Ma et al. 2010).

For other food source, derived polysaccharides were also documented as an active anticancer agent. For example, a RG-II-type polysaccharide extracted from green tea inhibited tumor metastasis, and the effect was related to activation of macrophages and NK cells (Park et al. 2017). The *Ganoderma atrum* polysaccharide (PSG-1) predominantly suppressed the tumor growth in tumor-bearing mice through mitochondria-mediated apoptotic pathways, cAMP/PKA signaling pathway, and PKC pathway (Li et al. 2011b). PSG-1 enhanced immunity, and antitumor effects might be related to their potential in promoting the production of TNF-α in tumor-bearing mice. The proposed signaling mechanism might be as follows: PSG-1 acted on the TLR4 receptors on macrophages, signaled through the p38 MAPK pathway, and then activated NF-κB that initiated the release of TNF-α (Li et al. 2011a; Qiang et al. 2015; Zhang et al. 2013). Ginseng leaves polysaccharide inhibited tumor metastasis via macrophage and NK cell activation (Shin et al. 2017). Intake of *Dendrobium candidum* polysaccharides significantly reduced the tumor weight and increased the levels of serum cytokines (IL-2 and TNF-α) in S180-bearing mice than that of without polysaccharide treatments (Jin et al. 2010). Luo and Fan proposed that the antitumor properties of *Dendrobium denneanum* polysaccharides were attributed to their excellent immune-enhancing and antioxidant activities (Fan and Luo 2011). Long et al. demonstrated an obvious immunoregulatory effect of *Polygonatum sibiricum* polysaccharides on tumor-bearing mice via TLR4-MAPK/NF-κB signaling pathways (Long et al. 2018). A sulfated polysaccharide extracted from *Undaria pinnatifida* showed a strong anticancer effect by 7, 12-dimethylbenz [a]anthracene (DMBA)-induced breast cancer rat model, which might be attributed to immunomodulatory ability and regulating abnormal sex hormone levels (Han et al. 2016). Liu et al. investigated the antitumor effect of tea polysaccharides on colitis-associated cancer in azoxymethane/dextran sulfate sodium (AOM/DSS) mice model. Results showed that tea polysaccharides inhibited the activation of STAT3 and the levels of downstream protein expression such as MMP2, cyclin-DI, surviving, and VEGF. Confirmed by the in vitro noncontact co-cultured cell system, tea polysaccharides were demonstrated to inhibited colitis-associated colorectal cancer via suppressing interleukin-6/signal transducer and activator of transcription (STAT)-3 signaling pathway (Liu et al. 2018). A water extracted polysaccharide from *Lentinus edodes* exerted direct antitumor effects by inducing of cell apoptosis via both reactive oxygen species (ROS)-mediated and TNF-α-mediated pathways (Wang et al. 2017a).

Besides, food polysaccharide combination with chemotherapeutics is regarded as a promising method for cancer therapy strategy. With the combination treatment of polysaccharides, an enhanced efficiency of CTX and relieved side effects caused by chemotherapy was documented (Li et al. 2011a; Zong et al. 2018).

On the other hand, microbial dysbiosis has been found in tumor genesis. Li et al. evidenced that the tumor-suppressive activity of *Ganoderma lucidum* polysaccharides was not only attributed to the activation of macrophages but also the modulatory effect of gut microbiota (Li et al. 2018a). As the most abundant metabolites of polysaccharides, SCFAs were considered to reflect the alteration of the composition of gut microbiota. SCFAs, especially butyrate, arising in the large bowel during

polysaccharide fermentation by bacterial were considered to take a vital part in its antitumor function in the colon. Three proposed aspects of polysaccharides reduced the risk of cancer were ameliorating leaky gut, avoiding hypernutrition, and reinforcing immune balance, as indicated by Liu et al (2019a).

Presently, several polysaccharides and polysaccharide conjugates (polysaccharide-protein and polysaccharide-peptide complexes), such as lentinan, schizophyllan, krestin, and grifolan, have been commercialized in clinical therapy of patients with anticancer therapy (Zhang et al. 2007). The antitumor effect of polysaccharides and the possible mechanisms have been studied extensively and reviewed (Chen and Huang 2018; Liu et al. 2015b, 2019a; Meng et al. 2016; Reshetnikov and Tan 2001; Zhang et al. 2007; Zong et al. 2012).

Based on the previous literatures, the proposed antitumor mechanisms were summarized as follows: (1) inhibition of the oncogenesis and prevention of tumor metastasis, (2) induction of apoptosis of tumor cells, (3) enhancement of host immune system, (4) improvement of oxidative stress, and (5) modulation of gut microbiome composition.

29.4.3 Antioxidant Effect

Oxidative stress arises either from excessive production of free radical species, a reduction of endogenous antioxidant defense capacity, or both. Lipid peroxidation is a destructive process in which oxidants react with lipids in cell membranes, leading to the damage of cell. The antioxidant enzymes play a key role in in vivo antioxidant system. Previous studies had demonstrated that the antioxidant enzymes could defense against the formation of ROS and transform active oxygen molecules into nontoxic compounds so as to reduce the oxidative stress. Specifically, superoxide dismutase (SOD), total anti-oxidizing capability (T-AOC), and glutathione peroxidase (GSH-Px) have been commonly chosen to be biomarkers in evaluation of oxidative stress. Among them, SOD is a kind of natural cellular antioxidant enzyme to scavenge free radicals and maintain cell metabolic balance. The prevention of GSH-Px against oxidative stress, on the other hand, is reduction of hydrogen peroxide and lipid peroxides into water and molecular oxygen. In the case of T-AOC, this parameter reflects the capacity of nonenzymatic antioxidant defense system (Mansour 2000). As for the lipid peroxidation, malondialdehyde (MDA) and lipid peroxidase (LPO) is generally regarded as sensitive indicator for evaluation of antioxidant abilities against oxidative stress as well (Liqin et al. 2015). Polysaccharide could obviously increase the levels of antioxidant enzymes and alleviate oxidative stress and lipid peroxidation in serum and organs (heart, liver, brain, renal, intestine, etc.). For example, the polysaccharides from plants and mushrooms, like *Ligusticum chuanxiong* (Chao et al. 2017), *Arctium lappa* (Liu et al. 2014), *Hericium erinaceus* (Han et al. 2013), *Lepista sordida* (Zhong et al. 2013), *Agaricus bisporus* (Li et al. 2018), Radix *Cyathulae officinalis* Kuan (Han et al. 2015), litchi pulp (Fei et al. 2016), aloe vera gel (Gaurav et al. 2014), *Dendrobium officinale* (Huang et al. 2015), oat β-glucan (Błaszczyk et al. 2015), and others, had a

beneficial effect on antioxidant enzymes of SOD, CAT, GSH-Px, and T-AOC and reduced MDA and LPO levels. An upregulated mRNA expression levels of antioxidant enzymes, including Cu, Zn-SOD, Mn-SOD, CAT, glutathione peroxidase 1 (GPx), thioredoxin 1, and thioredoxin 2 in organs including the liver, heart, and brain, were observed after oral administration of *Chuanminshenviolaceum* polysaccharides (Jing et al. 2017). Besides, Zhong et al. demonstrated that *Ganoderma lucidum* polysaccharide increased the expression of manganese superoxide dismutase (Mn-SOD), an important cellular antioxidant enzyme. They also found that polysaccharide inhibited the translocation and expression of p47phox, a core regulatory subunit of NADPH oxidase, indicating the antioxidant function of polysaccharide might be partially attributed to alleviate the NADPH oxidase-related ROS production pathway (Zhong et al. 2015). Jiang et al. pointed out that the improvement of oxidative stress by *Coptis chinensis* polysaccharide was closely related to JNK/IRS1/PI3K pathway (Jiang et al. 2015).

On the other hand, accumulation of ROS would activate mitochondrial and ER stress, triggering abnormalities, such as apoptosis and necrosis. *Ganoderma lucidum* polysaccharide reduced the ratio of Bax/Bcl-2 and inhibited the release of cytochrome c, in addition to reduce the expression of ER stress biomarkers including 78 kDa glucose-regulated protein (GRP78), CCAAT/enhancer-binding protein (C/EBP)-homologous protein (CHOP), and caspase-12 and inhibit the activation of JNK. These findings indicated *Ganoderma lucidum* polysaccharide inhibited the oxidative stress-induced apoptosis by a mitochondria-dependent pathway and alleviating ER stress (Zhong et al. 2015).

29.4.4 Antidiabetic Effect

Diabetes mellitus (DM) is a worldwide concern and obviously influences the quality of human life. It is an impaired carbohydrate, fat, and protein metabolic syndrome induced by insufficient insulin secretion or decreased tissue sensitivity to insulin. Generally, there are two types of DM, type 1 DM (T1DM) and type 2 DM (T2DM), withT2DM as the dominant which accounting for around 90% of DM (Skyler and Oddo 2002). For T2DM, insulin resistance (IR) and pancreatic β-cell dysfunction are the major causes, and an individual was diagnosed T2DM with the fasting blood glucose (FBG) $\geq$7.0 mmol/L (126 mg/dL) or 2 h oral glucose tolerance test of $\geq$11.1 mmol/L (200 mg/dL) (Würsch and Pi-Sunyer 1997). The effect of dietary polysaccharides on glucose control was mainly achieved by altering its small intestinal transit time (SBTT), preventing the carbohydrate from digestive enzyme suppression, and restraining these enzymes (Qin et al. 2012). Xiao et al. identified that the main antidiabetic polysaccharide fraction in *Ganoderma lucidum* polysaccharides was a low molecular weight β-heteropolysaccharide of 15.9 kDa. Its mechanism was probably associated with the downregulation of the hepatic glucose-regulated enzyme through AMPK activation and improvement of insulin resistance, as well as a decrease in epididymal fat/body weight ratio (Xiao et al. 2017). Wang et al. found that *Angelica sinensis* polysaccharide improved insulin

resistance via regulating the related metabolic enzymes and activating the PI3K/Akt pathway (Wang et al. 2016b). Likewise, Li et al. observed that PI3K/Akt pathway acted as the hypoglycemic mechanism of tea polysaccharides in T2DM mice model (Li et al. 2015).

Consumption of polysaccharides may add to the satiety and reduce hunger attributed to their huge volume and relative low energy density, in addition to affect the secretion of intestinal hormones or peptides. In addition, polysaccharide-rich foods can also enhance the satiety through long-term chewing, which will affect the amount and velocity of food intake. Meanwhile, they can promote the production of saliva and gastric acid through enhancing gastric distension (Trompette et al. 2014).

Diabetic animals feeding with the polysaccharide intervention could increase levels of gut *Firmicutes* and decrease levels of *Bacteroidetes* and alleviated diabetes. A recent report in gut provided the evidence of a high molecular weight polysaccharide (>300 kDa) from *Hirsutella sinensis* prevented diet-induced obesity and type 2 diabetes by modulating the composition of gut microbiota, of which selectively promoted the growth of gut bacterium *Parabacteroides goldsteinii*. Further oral treatment of obese mice with live *Parabacteroides goldsteinii* prevented body weight gain, improved intestinal integrity, and reduced inflammation and insulin resistance. These results proved that *Hirsutella sinensis* polysaccharide and *Parabacteroides goldsteinii* acted as novel prebiotics and probiotics that may be applied to obesity and type 2 diabetes (Wu et al. 2019).

On the other hand, polysaccharides from various edible sources, such as *Plantago asiatica* L. seeds, *Cyclocarya paliurus* leaves, *Ganoderma atrum*, *Dendrobium officinale*, fermented *Momordica charantia* L., *Astragalus membranaceus*, and others, have been confirmed to improve production of SCFAs, which were beneficial to the diabetics (Gao et al. 2018; Liu et al. 2019b; Nie et al. 2019; Zhu et al. 2016a). For example, butyrate prevented and inhibited colon carcinogenesis, protected against oxidative stress of mucosal, decreased inflammation, and enhanced the colonic defense barrier in diabetes (Wu and Wu 2012). The regulation of blood glucose concentrations may involve several positive effects exerted by SCFA production of polysaccharide occurring at different levels: (1) inhibition of the inflammatory state that decreased insulin resistance, (2) upregulation of GLP-1 secretion that stimulates insulin release, and (3) enhancement of β-cell function that results from improvement of glucose homeostasis.

Additionally, it was common to know that T2DM caused a complex metabolic problems and endocrine disorder. Specifically, a metabolomic approach was conducted to investigate the effect of polysaccharide on metabolites in type 2 diabetic rats. Zhu et al. investigated the effect of *Ganoderma atrum* polysaccharide on serum metabolites using ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) and identified eight potential biomarkers (methionine, dTMP, D-tryptophan, 5-hydroxy-indoleacetaldehyde, glycocholic acid, biliverdin IX, malate, and taurocholic acid). In addition, biological pathways and processes, including metabolism of carbohydrates, fatty acid biosynthesis, glycolysis and gluconeogenesis, and metabolism of lipids and lipoproteins,

are significantly changed by PSG-1 treatment (Zhu et al. 2016b). Nie et al. investigated the molecular mechanisms of arabinoxylan at the point view of urine metabolomics by UPLC-Triple-TOF/MS. Results suggested that TCA cycle, tryptophan metabolism, lipid and ketone body's metabolism, taurine and hypotaurine metabolism, lysine metabolism, and BCAA metabolism were significantly improved by arabinoxylan treatment. Besides, arabinoxylan may exert prebiotic effect by upregulating serotonin and melatonin biosynthesis and spermine biosynthesis pathway, as well as decreasing choline level (Nie et al. 2018a).

The possible mechanisms by which the polysaccharides were involved in the development of diabetes included energy metabolism, inflammation, innate immune system, and bowel function of the intestinal barrier in microbiota. Based on gastrointestinal process, polysaccharides could alleviate DM through the mechanisms of action of gastrointestinal viscosity, gastrointestinal satiety, large intestinal fermentation, and gastrointestinal anti-inflammation (Hu et al. 2018).

29.4.5 Renal Protective Effect

The biological effects of polysaccharides have been investigated previously in some animal models of kidney diseases, such as diabetic nephropathy; 5/6 nephrectomy; chemical-induced renal damages, like adenine, cadmium, and potassium bromated (KBrO3); and other renal injury models. Mechanisms of renal prevent or protective actions have been demonstrated for some polysaccharides. The effects were primarily related to anti-inflammation, anti-oxidation, immune-regulation, anti-fibrosis, and improvement of metabolic disturbance effects.

29.4.5.1 Diabetic Nephropathy

A glucan-rich polysaccharide from *Pleurotus sajor-caju* (Fr.) Singer improved the glucose tolerance, attenuated hyperglycemia, and hyperinsulinemia in diabetes mice with a high-fat diet through upregulating the adiponectin and GLUT-4 gene expressions, in addition to downregulating the expression inflammatory factor (Kanagasabapathy et al. 2012). The renoprotective function of *Ophiopogon japonicus* polysaccharide was related to reduction of hyperglycemia, which attenuated serum albumin concentration as well as downregulated TGF-β1expression and connective tissue growth factor (CTGF) in diabetic glomeruli (Wang et al. 2015b). *Inonotus obliquus* polysaccharide could restore the integrity of glomerular capsules and increase the amount of glomerular mesangial cells in glucolipotoxicity-induced renal fibrosis (Chou et al. 2016). *Lycium barbarum* polysaccharides improved the renal function and alleviated the inflammatory response in the kidneys of diabetic rabbits. It was worth noting that the prevention effect of polysaccharides on renal function was better than that of treatment effect (Zhao et al. 2016).

29.4.5.2 Chronic Renal Failure (CRF)

CRF is a progressive renal injury disease, and the incidence is rising markedly in recent years. Chinese chive polysaccharides were able to improve the kidney

functions in adenine-induced CRF, and the effect might be associated with its antioxidant, anti-inflammatory, and anti-fibrosis abilities (Li et al. 2018b). Wang et al. investigated fucoidan derivatives on chronic kidney disease in rats and revealed that substituted functional groups and molecular weight took a crucial part in protecting the kidney. The mechanisms were related to an alleviation of renal tubules, interstitium, and mesangial areas mediated by replacing electronegative content of the glomerular cells and inhibition of cell proliferation, in addition to their antioxidant activity (Wang et al. 2012). A purified *Laminaria japonica* polysaccharide significantly inhibited the progression of vascular calcification that was associated with preventing osteoblastic differentiation of vascular smooth muscle cells in adenine-induced CRF mice (Li et al. 2018c).

On the other hand, CRF is also associated with the systemic effects of malnutrition and muscle wasting. *Astragalus* polysaccharides were reported to ameliorate muscle mass and protein metabolism. The levels of serum pro-inflammatory factors and oxidative index (MDA and SOD) were restored by the polysaccharide treatment. The potential mechanism of muscle wasting was involved in protein anabolism (Akt/mTOR), ubiquitin-proteasome pathway, and autophagy signaling pathways (Geng et al. 2017; Lu et al. 2016).

29.4.5.3 Acute Kidney Injury (AKI)

Acute renal damage is increasing prevalent with a severe morbidity and mortality. Acute kidney failure is associated with KBrO3 or glycerol or ischemia reperfusion injury. Polysaccharides were showed to have a protective effect against AKI. A low molecular weight fucoidan ameliorated ischemia reperfusion injury caused AKI via inhibiting the activation of MAPK pathways, especially JNK/p38-MAPK (Chen et al. 2013). A low molecular weight sulfated polysaccharide fraction extracted from *Laminaria japonica* had a protective effect on glycerol-induced AKI, and the fraction contained higher amounts of fucose, and sulfate showed a better kidney protective activity (Li et al. 2017d). Comet assay, a method to evaluation of DNA damage/repair and genotoxicity testing, was conducted to investigate the DNA damage in the kidney. Results clearly showed that pretreatment of *Lycium barbarum* polysaccharides reduced the extent of KBrO3-induced DNA damage (Li et al. 2017).

29.4.5.4 Other Renal Injury Rat Models

Shen et al. reported that *Potentilla anserina* polysaccharide remarkably improved redox homeostasis in Cd-induced mice. It attenuated the mitochondria dysfunction, degeneration, and fibrosis of the kidney and exhibited anti-apoptosis activity via regulating both mitochondria-mediated intrinsic apoptotic pathway and the death receptor-initiated extrinsic pathway (Shen et al. 2017). Chiu et al. revealed that pretreatment of *Cordyceps sobolifera* polysaccharide improved LPS-induced kidney abnormalities and protected against LPS-triggered oxidative stress and inflammatory response (Chiu et al. 2014).

29.4.6 Liver Protective Effect

29.4.6.1 Acute Liver Injury Models

Acute liver damage is hepatic disorder caused by acute severe injuries induced by toxin chemicals, viral infection, metabolic disorder, and ischemic injury, leading to the functional loss of liver cells within 1–4 weeks. For evaluation of the injury of the liver, the activities of hepatic biomarker enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), in serum were considered as the indicators. Various literatures had revealed that polysaccharide could effectively lower the CCl_4-caused increases of serum ALT, AST, ALP, and LDH activities and hepatic MDA level and antagonize the decreases in antioxidant SOD and GSH activities caused by CCl_4. Polysaccharides from various dietary sources, such as apple (Yang et al. 2013), soybean (Sun et al. 2018), tea (Sun et al. 2013; Wang et al. 2014a), *Lycium barbarum* (Gan et al. 2018; Xiao et al. 2012), *Astragalus* (Hamid et al. 2017), *Rhodiola rosea* (Xu et al. 2018), and *Oudemansiella radicata* (Liu et al. 2017b), markedly attenuated liver injury through their anti-inflammatory and antioxidant actions. Xiao et al. revealed that *Lycium barbarum* polysaccharide promoted liver regeneration after CCl_4 treatment and attenuated hepatic inflammation through downregulation of pro-inflammatory mediators and chemokines. In addition, the protective activity was partly through the downregulation of NF-κB activity (Xiao et al. 2012). Recently, Gan et al. pointed out that *Lycium barbarum* polysaccharide alleviated CCl4-induced oxidative injury and inflammatory response and improved the liver fibrosis might be via inhibiting TLRs/NF-kB pathway (Gan et al. 2018).

Selenium (Se) is a very important trace element, and its combination with polysaccharide was reported to enhance the bioactivity of polysaccharide. Hamid et al. found that selenizing *Astragalus* polysaccharides showed obviously protective effects against CCl_4-induced liver injury and fibrosis was more preferable to both *Astragalus* polysaccharides and sodium selenite given alone. The protective effect was via inhibiting hepatic oxidative stress, inflammatory response, and fibrogenesis and inducing cell apoptosis (Hamid et al. 2017). Likewise, Gao et al. found selenylation modification of Chinese angelica polysaccharide significantly enhanced antioxidative and hepatoprotective function, and the mechanisms were suppressing the MAPK pathway (Gao et al. 2017). Besides, Se-containing tea polysaccharide could ameliorate the high fructose-induced insulin resistance and hepatic oxidative injury (Ren et al. 2015).

Besides, other chronic liver injury mice models were also conducted to investigate the protective effect of polysaccharide. *Angelica sinensis* polysaccharide markedly alleviated acetaminophen (APAP)-induced acute liver injury via amelioration of lipid peroxidation and oxidative stress, in addition to the inhibition of hepatic apoptosis (Cao et al. 2018). This polysaccharide could also attenuate concanavalin A (Con A)-induced liver failure through caspase-3-dependent apoptosis by caspase-8 and JNK-mediated pathway and inhibiting the activation of IL-6/STAT3 and NF-κB signaling pathways (Wang et al. 2016c). Polysaccharides from *Sophorae tonkinensis* Radix significantly attenuated APAP-induced hepatic

oxidative damage through inhibiting ROS generation and increasing the levels of antioxidant enzymes in the liver (Cai et al. 2018). A beneficial effect of sea buckthorn berries polysaccharide on LPS/d-GalN-induced acute liver failure was reported to be accompanied by a downregulation expression of TLR4, inhibition activation of ERK, JNK and p-38 MAPK, and NF-κB, indicating a suppression of TLR4-NF-κB signaling pathway involved (Liu et al. 2015a). Polysaccharide from stem of *Codonopsis pilosula* played a vital role in the protection against ischemia/reperfusion (I/R)-induced renal injury, and its renoprotective effect was probably mediated by inhibiting the release of pro-inflammatory cytokine (TNF-α) (Li et al. 2012).

29.4.6.2 Chronic Liver Diseases

Alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are two kinds of the most common precursors of chronic liver disease. NAFLD is a hepatic manifestation of the metabolic syndrome that is associated with obesity, type 2 diabetes, and atherosclerosis. Schisandra polysaccharide significantly reduced the liver index and ameliorated lipid accumulation and fatty degeneration of hepatocytes in NAFLD mice. The effects may be mediated to a downregulation of LXRα/SREBP-1c/FAS/ACC and SREBP-2/HMGCR pathways in the liver (Wang et al. 2016a). ALD is a prevalent global health issue as the liver is the major organ responsible for the metabolism of alcohol and alcohol consumption is common in daily life. Many dietary polysaccharides had been evidenced to have a protective or preventive effect against alcohol-induced liver damage. Maca polysaccharide also dramatically increased the SOD, GSH-Px, and GST levels in alcohol mice and lightened inflammation induced by alcohol (Zhang et al. 2017b). *Triticum aestivum* sprout-derived polysaccharide significantly inhibited ethanol-induced cytochrome P450 2E1 (CYP2E1) activation and upregulated the expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and hemeoxygenase-1, in addition to downregulated NADPH oxidase genes in mice. Furthermore, the upregulation of Nrf2 was mediated by PI3K/Akt pathway. Similarly, this polysaccharide attenuated hepatic injury by modulation of caspase-3 and apoptosis-associated mitochondrial proteins as observed in other models (Nepali et al. 2017).

The metabolic disorder improved by polysaccharides and underlying metabolic pathways involved had been studied. Metabolomic approach was employed to analyze the alterations in metabolic profiles of serum and the liver. Wang et al. investigated the metabolism pathways of *Dendrobium huoshanense* polysaccharide on alleviating ethanol-induced liver injury using UHPLC/LTQ Orbitrap XL MS. Results reveled that *Dendrobium huoshanense* polysaccharide ameliorated the altered metabolic levels particularly involved in phosphocholine and L-Proline (Wang et al. 2015a). Hepatic proteomic analysis performed by two-dimensional difference gel electrophoresis coupled with MALDI-TOF/TOF-MS revealed that cystathionine β-synthase (Cbs) and D-lactate dehydrogenase (Ldhd) were two key proteins in the *Dendrobium huoshanense* polysaccharide intervention. They might help in preventing the abnormal hepatic methionine metabolism pathway and

decreasing the level of hepatic methylglyoxal generated from disordered metabolic pathways (Wang et al. 2014b).

29.5 Cross-References

▶ Dietary Fibers

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Dietary Fibers

30

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Abstract

Dietary fibers have become increasingly popular in recent years with various applications in the food and pharmaceutical industry industries. Dietary fibers are plant-based foods which are incompletely digested by digestive enzymes. The water-soluble fibers are prebiotics, which are fermented in the colon and have a beneficial role for gut microbiota. The insoluble fibers are not affected by digestive systems and provide bulking. Fiber intake has various benefits: it reduces appetite, lowers variance in blood sugar levels, reduces the risk of cardiovascular disease, lowers the risk of diabetes, balances intestinal pH, alleviates constipation, and facilitates regular defecation.

Keywords

Dietary fibers · Polysaccharides · Oligosaccharides · Inulin · Pectin

30.1 Introduction

Codex Committee on Nutrition and Foods for Special Dietary Uses agreed on a definition of dietary fiber (DF) in 2009 (Codex Alimentarius 2009). According to the definition, dietary fibers are the carbohydrate polymers consisting of ten or more monomeric units that are not digested in human small intestine. Dietary fibers can be:

- Naturally occurring carbohydrate polymers found in food.
- Carbohydrate polymers that are isolated from raw food material using some treatments (physical, enzymatical, chemical). These have health benefits which have been demonstrated by scientific evidence.
- Carbohydrate polymers that are synthetic and have health benefits which have been demonstrated by scientific evidence.

Even though the Codex Alimentarius definition of dietary fibers mentioned that they have ten or more monomeric units, there are some definitions considering carbohydrates having 3–9 monomeric units (oligosaccharides) can also be dietary fiber. European Union (EU) defines dietary fibers as "carbohydrate polymers consisting of three or more monomeric units, that are not digested/absorbed in the small intestine" (European Commission 2008). According to definition of American Association of Cereal Chemists International (AACCI 2011), "dietary fibers are the edible parts of plants (poly- and oligosaccharides, lignin, etc) that are resistant to

digestion/absorption in the small intestine, but can be completely or partially fermented in the colon." Health Canada (2012) also reported that dietary fibers consist of carbohydrates with three or more monomeric units.

Dietary fibers include primarily non-starch polysaccharides that are plant cell walls components such as cellulose, hemicelluloses, and pectins. They also include some other polysaccharides of plant or algal (gums and mucilages) and oligosaccharides (inulin) (Mudgil and Barak 2013). Resistant starches, fructo- and galacto-oligosaccharides, and modified celluloses are analogous carbohydrates that are considered as dietary fibers. Some synthetic carbohydrates such as polydextrose are also included in dietary fiber (Borderías et al. 2005).

In recent years, dietary fibers have received much attention because of their beneficial health effects such as reducing cholesterol, diabetes, and coronary heart disease, improvements in large bowel function, and reduction of postprandial blood glucose and insulin levels (Mudgil and Barak 2013). The physiological effects of dietary fibers are related to their physicochemical properties such as solubility, water-holding capacity, viscosity, etc. (Guillon and Champ 2000). Dietary fibers are divided into two categories based on solubility: soluble and insoluble fibers. Insoluble dietary fibers are mainly cell wall of plants such as cellulose, lignin, and hemicelluloses. On the other hand, soluble fibers are non-cellulosic polysaccharides such as pectin, gum, mucilage, β-glucan, etc. (Dai and Chau 2017). Insoluble fibers are responsible for the increase in fecal bulk and decrease in the intestinal transit time. Soluble fibers form gels and increase the viscosity of the contents of the gastrointestinal tract causing delayed gastric emptying. They are also are associated with reduction in glycemic response and plasma cholesterol (Mudgil and Barak 2013).

30.2 Bioactive Constituents

Cellulose, hemicelluloses, inulin, dextrin, oligosaccharides, resistant starch, pectin, β-glucan, glucomannan, guar gum, psyllium, and methylcellulose are the main examples of dietary fibers. Lignin and minor compounds such as waxes, cutin, saponins, polyphenols, phytates, and phytosterols can be extracted with poly- and oligosaccharides during fiber analysis procedures (Table 1). However, these compounds, except lignin, are not considered as dietary fiber (Mudgil and Barak 2013).

30.2.1 Cellulose

Cellulose is an insoluble fiber found naturally in nuts, whole wheat and grains, bran, seeds, edible brown rice, etc. It is the major cell wall component in plants and the most abundant polysaccharide found in nature. It is an unbranched linear polysaccharide with 10,000 glucose unit per molecule linked with β,1-4 glycosidic bonds. Cellulose is an insoluble fiber and resistant to digestive enzymes. However, 50% of cellulose can be fermented by gut microflora in the colon, and short-chain fatty acids

Table 1 Functional properties and main sources of dietary fibers, according to Bozzetto et al. (2018) and McRorie (2015)

Fibers	Functional properties			Main sources
	Viscosity	Solubility	Fermentation	
Bran	Low	Low	Low	Whole grain
Cellulose				Vegetables
Hemicellulose				Vegetables
Lignin				Seeds
Inulin	Low	High	High	Roots and tubers
Dextrin				Chemically altered wheat and corn starch
Oligosaccharides				Fruits, vegetables, legumes, grains
Resistant starch				Type I, whole grain; Type II, high-amylose maize starch, raw potato, and banana; Type III, cooked and cooled starchy foods; Type IV, chemically modified starches; Type V, amylose-lipid complex
Pectin	High	High	High	Fruits, vegetables, legumes
β-Glucan				Oat and barley
Glucomannan				Tuberous roots of the konjac plant
Guar gum				Leguminous seed plants (guar, locust bean), seaweed extracts (carrageenan, alginates), microbial gums (xanthan, gellan)
Psyllium	High	High	Low	Husks of ripe seeds from *Plantago ovata*
Methylcellulose				Food additive

can be produced (Mudgil and Barak 2013). Due to its water-binding capacity, cellulose causes an increase in fecal bulk (Dai and Chau 2017).

30.2.2 Hemicellulose

Hemicelluloses are the second most abundant polysaccharides after cellulose. Similar to cellulose, hemicelluloses are also one of the cell wall components. They are not chemically homogeneous like cellulose and include both linear and branched molecules consisting of different types of pentoses, hexoses, and uronic acids. These monosaccharides can be xylose, rhamnose, arabinose, glucose, mannose, galactose, and some uronic acids (4-*O*-methylglucuronic, D-glucuronic, and D-galacturonic acids). Hemicelluloses have lower molecular weight compared to cellulose (Hu et al. 2009). Hemicelluloses increase the hydration of the stool causing a promotion of bowel movements. Hemicelluloses prevent cholesterol absorption by binding cholesterol in the gut (Mudgil et al. 2012). Similar to cellulose,

hemicelluloses can be also digested by gut bacteria, and as a result short-chain fatty acids can be used as fuel by colon cells (Mudgil and Barak 2013). Hemicelluloses can be extracted by solubilization in aqueous alkali after removing the water-soluble and pectic polysaccharides.

30.2.3 Lignin

Lignin is not a polysaccharide but is considered as a dietary fiber. It is found in woody part of the foods and in outer layers of cereal grains as chemically bound to hemicelluloses in the plant cell wall.

30.2.4 Gums and Mucilages

Gums and mucilages are types of dietary fibers that are not found in plant cell wall, but produced in specialized secretory plant cells (Dhingra et al. 2012). They are highly branched polysaccharides and are used in foods as gelling, thickening, stabilizing, and emulsifying agents (Mudgil and Barak 2013). Gums are known as pathological products of plants formed after injury or as a result of unfavorable conditions (i.e., drought) by the breakdown of cell walls (extracellular). However, mucilages are normal products of plant metabolism and formed intracellularly (within the cell). Gum arabic, guar gum, locust bean gum, agar, carrageenan, and alginates can be given as examples of gums. Psyllium is an example of mucilages. They both have effect on reducing glucose and insulin levels and cholesterol (Mudgil et al. 2012).

30.2.5 β-Glucans

β-Glucans are the major component of the cell wall material in oats and barley grains and also present in wheat in small quantities. *They* are consisting of D-glucose monomers linked by β-glycosidic bonds. The macromolecular structure of β-glucan depends on the source. The simplest β-glucan is found among prokaryotes and eukaryotes, and it is linear and unbranched (β-(1,3)-D-glucan) (El Khoury et al. 2012). Branched structures consist of β-(1,3)- or β-(1,4)-glucan backbone with (1,2)- or (1,6)-linked branches (Barsanti et al. 2011). β-Glucans are water-soluble fibers and can form gel-like network in the upper gastrointestinal tract. The cholesterol lowering ability of β-glucans is considered to be related to their gelling capacity (Mudgil and Barak 2013).

30.2.6 Pectins

Pectins are the polysaccharides found in plant cell walls and outer skin and skin of fruits and vegetables. The main constituent of pectin is galacturonic acid. They

are soluble in hot water and can form gels upon cooling; therefore they are used as a thickening agent in foods. Similar to β-glucans, the gelling property of pectins is responsible for cholesterol lowering ability (Mudgil and Barak 2013).

30.2.7 Resistant Starch

Digestible starches can be hydrolyzed by digestive enzymes to give glucose to be absorbed (Nugent 2005); however, a fraction of starch cannot be digested in the body and defined as resistant starch (RS). European Food-Linked Agro-Industrial Research-Concerted Action on Resistant Starch (EURESTA) defined RS as the "total amount of starch and the products of starch degradation that resists digestion in the small intestine of healthy individuals" (Öztürk and Köksel 2014). RS can be classified into five groups (Okumus et al. 2018): RS1, starches which are physically inaccessible to digestion as they are entrapped within whole or partly milled grains; RS2, native starch granules that are found in raw potatoes, green bananas, and high-amylose corn starch; RS3, retrograded starches found in cooked-cooled potato, in bread, and in corn flakes; RS4, chemically modified starches to obtain resistance to enzyme digestion; and RS5, amylose-lipid complexes that are resistant to enzymatic digestion. Resistant starches have physiological functions similar to those of dietary fibers. They are insoluble fibers; however, they act as soluble fibers. There are several studies investigating that RS can reduce the risk of diabetes, obesity, and high cholesterol. Fermentation of resistant starches results in formation of short-chain fatty acid that has inhibition effect on the development of colonic cancer cells (Haralampu 2000; Huth et al. 2000; Öztürk and Köksel 2014; Wollowski et al. 2001).

30.2.8 Nondigestible Oligosaccharides

Nondigestible oligosaccharides with 3–10 monomeric units are found in fruits, vegetables, and cereals. They can also be synthesized from mono-, di-, and polysaccharides by chemical and enzymatic processes. Fructooligosaccharides, gluco-oligosaccharides, and inulin are examples of nondigestible oligosaccharides. They can be fermented in the large intestine by beneficial bacteria causing the formation of short-chain fatty acids which are beneficial for colon health (Jian et al. 2013).

30.2.9 Synthetic Carbohydrate Compounds

Synthetically prepared methylcellulose, hydroxy-propylmethylcellulose, polydextrose, and resistant dextrins are also considered as dietary fibers as they are also nondigestible. Unlike cellulose methylcellulose and hydroxy-propylmethylcellulose are soluble fibers but hardly fermented in the large intestine (Mudgil and Barak 2013).

Polydextrose and resistant dextrins can be partially fermented in the colon and have bulking ability (Craig et al. 2000; DeVries 2003).

30.3 Bioactivities (Animal Aspects)

30.3.1 Anticancer Activity

Environmental aspects such as diet and physical activity and genetic deviation are the two main factors effecting the colon cancer formation (Zeng et al. 2014). Several animal studies have suggested that there is a relationship between high dietary fiber intake and low prevalence of colon cancer (Caderni et al. 2001; Cho et al. 2012; Clarke et al. 2008; Conlon et al. 2012; Kameue et al. 2004; Le Leu et al. 2007). Most of these benefits can be associated with short-chain fatty acid production during the fermentation of dietary fibers in the colon. These short-chain fatty acids are the energy source of colonocytes and prevent tumor growth. The major short-chain fatty acids are acetic acid, propionic acid, and butyric acid. Among these fatty acids, butyric acid is the critical one in maintaining colon health (Macfarlane and Macfarlane 2011). The preventative effect of dietary fibers may be attributed to the reduced contact time of carcinogens within the intestinal lumen and to the production of the metabolites produced during the metabolism of fibers in the body (Zeng et al. 2014).

30.3.2 Antidiabetic Activity

Dietary fibers have been associated with improved postprandial glucose and insulin responses. There are many studies investigating the reduction of postprandial glucose responses after fiber-rich meal consumption (Fotschki et al. 2014; Grover et al. 2011; Utrilla-Coello et al. 2013). The postprandial glucose reducing effect of soluble fibers is due to the gel forming ability of soluble fibers (Cassidy et al. 2018). On the other hand, consumption of insoluble fibers reduces the risk of type 2 diabetes (Mudgil and Barak 2013).

30.3.3 Antihypercholesterolemic Effect

Bile acids are the end products of cholesterol catabolism and are synthesized from cholesterol in the liver. After their function they are reabsorbed and returned to the liver (Chiang 2013; Dawson et al. 2009). Dietary fibers have been reported to bind bile salts in the duodenum, and this results in the synthesis of additional bile salts from cholesterol which lowers blood cholesterol. The decrease in the blood cholesterol was also observed in several animal studies (Agyekum and Nyachoti 2017; Bennani-Kabchi et al. 2000; Gallaher et al. 2000; Moharib and El-Batran 2008; Terpstra et al. 2000).

30.4 Bioavailability and Metabolism

Epidemiological data suggest that populations subsisting on high-fiber diets are free of the diseases of Western civilization, among them are coronary heart disease and cancers. Underlying mechanisms of action are worth investigating regarding protective effects on the cardiovascular system, and bioactive components in fibers should be isolated and identified (Tang et al. 2017). Studies in animals and humans show that each type of fiber has specific effects, i.e., in man bran has no effect on serum lipids, but pectin lowers cholesterol levels. In animals fed atherogenic diets, alfalfa and pectin exert some protection, while cellulose does not. Kritchevsky considers that in order to understand the effects of different dietary fibers, it is necessary to assess the mechanism(s) of action of each chemical component of a given fiber (Kritchevsky and Story 1978).

Among fiber isolates, gelling and mucilaginous fibers, like pectins and guar gum, lower circulating lipids in humans and increase excretion of bile acids resulting from the fecal metabolism of cholesterol. These fiber components influence luminal solubility of lipids and the lymphatic absorption of cholesterol and triglycerides. The direct effects on lipid absorption could explain the hypolipidemic actions and secondary long-term consequence on hepatic and peripheral lipoprotein metabolism (Vahouny 1982).

β-glucans are linear unbranched polysaccharides found in natural sources, especially mushrooms. They act as dietary fibers in the digestive tract. Their structure enables interaction with innate immunity receptors. There are two forms of β-glucans, insoluble and soluble, which are able to interact in the bowel with lipids and biliary salts and determine reduction of cholesterol levels (Sima et al. 2018). Barley-derived β-glucans are viscous dietary fibers, which delay gastric emptying and cause gastric distension, release appetite-related hormones, and slow glycemic responses (Aoe et al. 2014). Yeast-derived beta-glucans have an immunomodulatory function by stimulating type 2T helper cells, which mitigate type 1T helper cells. Through this modulation results a balance between the two lymphoid systems and a decrease of the intestinal inflammatory process. β-Glucans can also stimulate phagocytosis, which protects against inflammation, sepsis, and infections (Schwartz and Hadar 2014).

Based on observing robust positive effects of whole grain intake in 11 major prospective studies, Lillioja et al. provide compelling evidence that increasing whole grain intake is an important public health issue. They consider that whole grain intake is often equated with fiber intake, regarded as inert material, while the most highly concentrated source of nutrients in cereals is aleurone, a live plant tissue that binds magnesium, zinc, and ferulic acid, responsible for health effects. They also describe three separate benefits attributable to whole grain fibers: the stool bulking effect, regulating the access by microbial digestive enzymes; the creation of gels by cell wall polysaccharides, especially pectins and β-glucan; and the fermentation of polysaccharides to volatile fatty acids with huge direct health benefits (Lillioja et al. 2013). In the past decades, resistant starches (RS) have also been used for their health benefits. They stay undigested in the small intestine but undergo fermentation

in the large intestine. RS restrict gluconeogenesis and enhance glycogenesis, maintaining glucose and lipid homeostasis and thus improving pancreatic function (Meenu and Xu 2018).

Fardet et al. comment on the synergy that occurs when more nutrients affect different pathways of the same disease process or when their effects are summed. Summed effects could occur between natural chemicals in whole grains which include selenium, manganese, zinc, iron, magnesium, tocotrienols, B vitamins, carotenoids, anthocyanins, choline, and fermentable polysaccharides. Both combined and individual effects must be understood (Fardet 2019).

Development of atherosclerosis has been linked to infections. Certain microbial ecosystems residing in the human body that contribute to metabolic and cardiovascular diseases have been identified. Microbiota affects atherogenesis by three pathways: local or distant infections causing harmful inflammatory responses with consecutive plaque rupture; metabolism of cholesterol and lipids by gut microbiota affecting the development of atherosclerotic plaques; and diet-specific components metabolized by gut microbiota (Jonsson and Backhed 2017). However, other authors have a different point of view. The study of Bindels et al. demonstrated that metabolic benefits exerted by dietary RS occur independently of the microbiota, especially improvements in insulin levels, and could involve alterations in the bile acid cycle and also adipose tissue immune modulation (Bindels et al. 2017). Mannan oligosaccharides (MOS) proved very effective at reducing hyperlipidemia and inflammation. The effect of dietary MOS on atherosclerosis development was studied by Hoving et al. in hyperlipidemic ApoE∗3-Leiden.CETP (E3L.CETP) mice, a well-known model for human-like lipoprotein metabolism. MOS may improve inflammation via interaction and modification of gut microbiota, the authors suggest. They found that MOS modulated the gut microbiota composition and activity associated with increased fecal BA excretion, which could explain lowered plasma total cholesterol levels and stopping of atherosclerosis progression (Hoving et al. 2018).

Dysbiotic gut ecosystems increase production of endogenous ethanol, disrupting the integrity of the intestinal barrier with increased accumulation of hepatic fat and production of inflammatory markers (Poeta et al. 2017). Gut-derived lipopolysaccharides contribute to increased hepatic oxidative stress and inflammation, fibrosis, and insulin resistance, with further alteration of intestinal barrier integrity. These dysfunctionalities of the gut-liver axis determine the phenotype of fatty liver (Saez-Lara et al. 2016).

Myoinositol, a member of the vitamin B complex, stimulates contractility of the gastrointestinal tract and has a key role in cell membrane potential maintenance, intracellular signaling, and calcium concentration control (Ciacci et al. 2011).

Soluble fibers, like pectin, resistant starches, gums, and inulin are fermented by colonic microbiota, producing short-chain fatty acids (acetate, propionate, and butyrate). Short-chain fatty acids regulate appetite by their anorexigenic effect, slowing gastrointestinal motility and controlling nutrient absorption. They modulate the release of appetite-related hormones (cholecystokinin, ghrelin, glucagon-like peptide 1, and peptide YY) (El Khoury et al. 2012).

30.5 Benefits (Human Studies)

30.5.1 Cardiovascular Disease

Cardiovascular disease (CVD) is a heavy social burden in many countries, being a category of chronic noncommunicable diseases that cause high mortality (Chan et al. 2013). Hypertension, dyslipidemia, obesity, and type 2 diabetes mellitus are the main components of cardiovascular risk (Kones and Rumana 2014). The "Western" diet, low in fiber and high in fats, sugars, and animal proteins, is the main factor that contributes to inflammation and metabolic syndrome. The most important strategy to treat atherosclerosis is lifestyle change, knowing the beneficial properties of various nutrients. In recent years, epidemiological and clinical studies have focused on the central role of diet plays in the prevention of atherosclerosis (Torres et al. 2015).

The cardioprotective effects of fibers are multiple, from anti-oxidation and anti-inflammation to attenuation of myocardial damage, modulation of enzyme activities, and regulation of blood pressure, glucose, and lipid profiles (Tang et al. 2017). These effects usually occur with the synergistic intervention of bioactive components like vitamins, minerals, and phytochemicals from vegetables. Soybeans, onions, sesame, tomatoes, celery, lettuce, and asparagus have great potential in cardiovascular prevention (Tang et al. 2017). Green leafy vegetables have been shown to significantly reduce the incidence of CVDs in a recent meta-analysis (Pollock 2016). In another study, different varieties of carrots demonstrated antithrombotic activities (Yamamoto et al. 2008). Broccoli, through sulforaphane present in this vegetable, has been found to protect the myocardium from ischemic reperfusion injury (Mukherjee et al. 2010).

Dietary fibers are characterized by physiochemical complexity of their individual components, and experimental, clinical, and epidemiological evidences confirm the role of these components in modifying cardiovascular risk factors.

An extensive meta-analysis of 15 cohort prospective studies included a large general population with low CVD risk (n = 1,409,014) and demonstrated that dietary fiber consumed increased amounts leads to reduced risk of mortality from CVD (Kim and Je 2014).

30.5.1.1 Dyslipidemia

Hypercholesterolemia is an important risk factor of cardiovascular disease, together with hypertension and diabetes. The search for new methods of preventing dyslipidemia is ongoing, and current therapies induce different side effects. Statins are the most common drugs administered for hypercholesterolemia, but statin-associated muscle pain lowers adherence to therapy. Soluble fiber, alone or in combination with plant sterols and stanols, red yeast rice, bergamot, berberine, or artichoke, might be considered as an alternative therapy, still in need of evidence for long-term safety and efficacy. It is considered that these nutraceuticals exert significant lipid-lowering activity and have pleiotropic actions like improvement of

endothelial dysfunction and arterial stiffness, together with anti-inflammatory and antioxidative properties (Banach et al. 2018).

It is a known fact that water-soluble dietary fiber significantly decreases serum cholesterol levels and may contribute to prevention of arterial thrombotic disease. Epidemiological studies have shown that vegetable consumption is inversely correlated to cardiovascular risk.

Wheat bran does not alter significantly lipoprotein distributions or serum lipids in humans. In contrast, dietary fibers from fruits and vegetables determine stronger responses, i.e., pectin, a viscous fiber decreases progression of IMT thickening (Wu et al. 2003).

CHD and stroke as evolution of atherosclerosis related to hyperlipidemia, hyperinsulinemia, and hypertension are strongly related to diet. In the study of Veldman et al., 20 male hyperlipidemic volunteers two randomized in groups received a pectin supplement (15 g/day) or placebo (15 g/day) for 4 weeks. Pectin supplementation caused significant decreases in total cholesterol, LDL, apolipoprotein A and B, and lipoprotein(a). In the plasma of the pectin-supplemented group, the development of more permeable fibrin networks believed to be less atherogenic was detected. It is believed that pectin modified network characteristics by a combination of its effects on the metabolism of fibrin, confirming the therapeutic effects of dietary intervention. This study also showed that fibrin network architecture is independent of plasma fibrinogen levels, contradicting what was previously thought (Veldman et al. 1997).

In order to determine the efficacy of Anticholest, an apple-pectin-guar soft drink in reducing elevated cholesterol levels, 33 participants (aged from 8 to 73 years) were divided into 3 groups receiving dosages of 1 cup every second day or of 1 cup or 2 cups a day. Anticholest significantly reduced TC, LDL-c, and TC/HDL-c, increasing HDL-c in a dose-dependent manner ($p < 0.01$). A significant decrease was also detected in TGL ($p < 0.05$). Anticholest appears to be an appropriate treatment for patients with increased cardiovascular risk, and the lack of side effects and its easy administration as viscous drink are essential for long-term compliance and management of chronic progressive conditions like atherosclerosis (Pirich et al. 1992).

Oats are a rich source of β-glucan, a viscous, soluble fiber known for its cholesterol-lowering properties. A meta-analysis of randomized controlled trials investigated the effect of diets enriched with oat β-glucan compared with controlled diets on LDL cholesterol, non-HDL cholesterol, and apoB. Data were pooled using the generic inverse-variance method with random effects models. Pooled analyses showed that the median dose of 3.5 g/d oat β-glucan significantly lowered LDL cholesterol (−0.19; 95% CI −0.23, −0.14 mmol/l, $p < 0.00001$), non-HDL cholesterol (−0.20; 95% CI −0.26, −0.15 mmol/l, $p < 0.00001$), and apoB (−0.03; 95% CI −0.05, −0.02 g/l, $P < 0.0001$) compared to control. Ho et al. consider that inclusion of oat-containing foods may be a strategy for achieving lipid targets in CVD prevention (Ho et al. 2016). The molecular weight (MW) of β-glucan contributes to viscosity, and individual genotype influences the cholesterol-lowering efficacy of β-glucan. In the study of Wang et al., HMW β-glucan rather than LMW

β-glucan reduced circulating TC in mildly hypercholesterolemic adults. Individuals carrying the CYP7A1SNP rs3808607-G allele were more responsive to the cholesterol-lowering effect of β-glucan with HMW (Wang et al. 2016).

The results of a study with bael leaf extract reported by Asghar et al. demonstrated its protective role in hypercholesterolemia. Bael leaf contains crude fiber (8.1 g% in the infant plant, 11.0 g% in the mature, and 9.5 g% in the ripen plant). Supplementation with crude extract decreased total cholesterol, triglycerides, LDL, and HDL in the study patients compared to control (Asghar et al. 2018).

Studies evaluating the effect of beans on cholesterol have focused on soybeans and other beans, peas, and seeds commonly consumed in Western countries. A meta-analysis of randomized controlled trials evaluating the effects of non-soy beans on blood lipids was conducted by Bazzano et al. From 140 relevant studies, 10 randomized clinical trials comparing a non-soy bean diet to control, with a duration of at least 3 weeks, were selected, representing a total of 268 participants. The decrease in total cholesterol in the intervention groups compared to control was −11.8 mg/dL (95% CI, −16.1 to −7.5) and of LDL −8.0 mg/dL (95% CI, −11.4 to −4.6). HDL cholesterol did not change significantly. The selected studies included participants with high, borderline high, and normal cholesterol levels who were not on cholesterol-lowering drugs. Total baseline cholesterol ranged from 199 to 294.6 mg/d. The majority of participants in the supplementation groups was represented by men and achieved lower total cholesterol and LDL levels than women. This meta-analysis represents the strongest evidence to date that consumption of non-soy beans lowers serum total and LDL cholesterol, contributing to decrease CVD risk (Bazzano et al. 2011).

Although current dietary guidelines recommend the consumption of three cups of dried beans per week, present consumption is much lower. Guenther et al. consider that consumption of starchy vegetables, mainly white potatoes, is much higher than recommended. They suggest dietary modification strategies with an increase in bean consumption targeted at CVD risk factor reduction (Guenther et al. 2010).

A total of 22,915 participants from the EPIC Norfolk study were included in a cross-sectional analysis which proved that mean fiber intake of 18.5 g/day lowers triglycerides and serum cholesterol levels (Wu et al. 2007).

30.5.1.2 Hypertension

In recent years, new bioactive components of foods have emerged to also control blood pressure levels, besides influencing other risk factors. Several studies suggest that beta-glucan, the soluble fiber from oat grains which contributes to reduction in plasma cholesterol, may also improve glycemia, insulin resistance, and weight loss. Although far fewer than the lipid studies, small trials with dietary fiber supplementation in hypertensive and pre-hypertensive subjects have shown that fiber intake decreases arterial blood pressure. This effect was first confirmed in research on hypertensive rats (Aleixandre and Miguel 2016).

A total of 18,433 subjects aged 18 years or older were included in an analysis aimed to explore the connection between dietary fiber intake and the risk of hypertension. Hypertension values were considered ≥130 mmHg systolic blood

pressure (SBP) and ≥ 80 mmHg diastolic blood pressure (DBP) according to the 2017 American College of Cardiology/American Heart Association Blood Pressure Guidelines. Recall interviews were used to obtain dietary fiber data from the National Health and Nutrition Examination Survey 2007–2014, and logistic regression models were applied to assess associations between consumption of cereal, vegetable, fruit fiber, and hypertension. Total fiber intake was associated in a nonlinear trend with the risk of hypertension, while the relation between cereal and vegetable fiber intakes with hypertension risk was linear. Fruit fiber consumption was not associated with a decreased risk of hypertension in US adults. The study concluded that the risk of hypertension gradually decreased as total dietary fiber intake increased up to 0.35 g/kg/day and that consumption of fiber-rich foods is advisable to prevent and control hypertension (Sun et al. 2018).

Another cross-sectional study on 111 T1DM patients with diabetes duration 18 +/− 9 years demonstrated that increased dietary fiber intake contributes to lower BP levels. BMI was 24.8 +/− 3.85 kg/m^2 and HbA1c 9.0 +/− 2.0%. Patients were randomized into two groups according to the American Diabetes Association (ADA) recommendations for fiber intake: < 14 g fiber/1000 kcal and >/= 14 g/1000 kcal. Increased daily mean total fiber intake of 19.8 ± 7.2 g/day (5.8 ± 2.4 g from soluble and 14.0 ± 5.3 g from insoluble fiber) determined significant decreases of BP levels. Furthermore, the authors reported an association between fiber type and BP. Dietary fibers originating from pulses (beans, lentils, peas) and fruit (banana, orange, grape, apple, papaya, pineapple, mango, and peach) were associated with the most significant decreases of BP, suggesting that fiber intake may favorably affect vasodilatation and oxidative stress. The authors consider that additional studies are needed to explain the mechanisms implied in the beneficial effects of fiber intake on BP levels (Beretta et al. 2018).

Lillioja et al. provide recommendations about whole grain consumption, consecutive to a meta-analysis of 11 major prospective studies from the United States that examined the effect of whole grain intake on the development of T2DM, CHD, and hypertension. Disease incidence was correlated to whole grain intake (grams/day). The risk of T2DM, CHD, and hypertension was significantly lower with higher whole grain cereal intakes. The authors consider that in order to maintain health, 40 g or more whole grains should be consumed daily, equivalent to a bowl of whole grain breakfast cereal. They also point at aleurone in bran, a critical grain component overlooked in favor of indigestible fiber. In millers' bran live aleurone cells are present in a concentration of 50% and store minerals, protein, and ferulic acid, a powerful antioxidant. Deficits of magnesium, zinc, and ferulic acid are involved in the development of chronic disease. Dieticians and physicians should be more concerned about the possible mechanisms by which aleurone exerts its effects on health, and also consumers should scan more carefully package labels on whole grain content. This change of attitude could generate enormous public health benefits (Lillioja et al. 2013).

A 6-month, open-label clinical trial enrolled 141 consecutive hypertensive and overweight patients, randomized to oral ingestion of either psyllium powder or guar gum 3.5 gr t.i.d., taken 20 min before the two main meals or to standard diet.

Ingestion of each fiber significantly improved BMI, fast plasma glucose and insulin, HbA1c, LDL-C, and apoB. Improvements in plasma TG concentration, SBP, and DBP were only exerted by the psyllium supplementation, compared to guar gum supplementation or standard diet (Cicero et al. 2007).

Data from the French ENNS 2006–2007 Nutrition and Health Survey that included 18–74-year-old participants demonstrated that dietary fiber and whole grains reduce systolic blood pressure (Vernay et al. 2012).

A cross-sectional study from the INTERnational study cohort on MAcro/micro-nutrients and blood Pressure (INTERMAP) made on 2195 US participants aged 40–59 years investigated the correlation between BP and total soluble and insoluble fiber intake. Aljuraiban et al. showed that an intake of total fibers of 6.8 g/ 1000 kcal lowers systolic blood pressure with 1.69 mmHg and an insoluble fiber intake of 4.6 g/ 1000 kcal with 1.81 mmHg (Aljuraiban et al. 2015).

Another large prospective study was made on 28,926 US health professional middle-aged and older women and revealed that consumption of 1–2 and 2–4 servings/day of whole grains lowers the risk of hypertension (Wang et al. 2007).

30.5.1.3 Diabetes

Type 2 diabetes (T2DM) has reached epidemic proportions in the United States and Western countries. Recent evidence suggests that prebiotics can modulate the gut microbiome, an important link in regulating lipid metabolism, blood glucose, and insulin sensitivity. Prebiotics are potential therapeutic strategies for prediabetes and T2DM (Alfa et al. 2018).

In a cross-sectional study on 4,399 Japanese type 2 diabetic patients, Fuji et al. demonstrated that increased dietary fiber intake was associated with better glycemic control and better CV risk reduction. Dietary fiber intake was determined using a brief self-administered diet history questionnaire. Improvements were observed in various parameters including insulin sensitivity and micro-inflammation, metabolic syndrome, blood pressure, and CKD. Furthermore, dietary fiber intake lowered the prevalence of albuminuria and improved estimated glomerular filtration rate after multivariate adjustments including protein intake (Fujii et al. 2013).

Another cross-sectional study of Ahola et al. found that dietary fiber was associated with lower mean blood glucose concentrations in patients with T1DM. Glycemic values were even lower when protein was replaced with other macronutrients and when fat substituted carbohydrate, after adjustments for fiber intake (Ahola et al. 2018).

Numerous human studies involving resistant starches have shown a decrease in postprandial blood glucose and insulin levels (Lattimer and Haub 2010). In a critical review on antidiabetic and anti-obesity effects of dietary resistant starch, Meenu and Xu concluded that resistant starches have many health benefits that are different from digestible starches. Numerous food products were fortified with RS to increase its dietary intake and exhibited positive impact on human health (Meenu and Xu 2018).

The STARCH trial was the first metabolic phenotyping that studied the effect of resistant starch on diabetes risk factors. It included 63 overweight or obese

participants (BMI $\geq$ 27 kg/m^2, weight $\leq$ 143 kg), with ages between 35 and 75 years with confirmed prediabetes. They were randomized to consume either 45 g/day of RS (RS = amylose) or amylopectin (control) for 12 weeks. The effect of RS on inflammatory markers and insulin sensitivity was evaluated. Energy expenditure, appetite, food intake, substrate oxidation, colonic microbial composition, fecal and plasma levels of short-chain fatty acids, and gut permeability were secondary outcomes. Dietary resistant starch (RS) improved gastrointestinal tract function, inflammatory markers, and insulin sensitivity significantly compared to amylopectin, lowering the risk for diabetes (Marlatt et al. 2018).

In patients with glucose intolerance or newly diagnosed T2DM, 4 weeks of dietary treatment with fortified rice containing RS improved endothelial function and reduced postprandial glucose and oxidative stress compared with control (Kwak et al. 2012).

Another study examined the effect of resistant starch on blood glucose, insulin levels, dyslipidemia, and humoral immune responses in healthy overweight subjects (over 120% ideal body weight) fed either 24 g/d RS or regular corn starch (CS) for 21 days. No significant changes in weights or other physical parameters occurred following intakes. However, there were significant decreases of total cholesterol (p <0.05), LDL cholesterol (p <0.05), and fasting serum glucose concentrations (p <0.05) in subjects supplemented RS. RS supplement also increased serum immunoglobulin G (IgG) concentrations. Insulin levels were unaffected. The supplementation was considered palatable with minimal bowel discomfort (Park et al. 2004).

Dainty et al. propose a dietary strategy for T2DM risk reduction by means of a diet with high-amylose maize resistant starch bagels that improves fasting insulin sensitivity in adults at increased risk of T2D (Dainty et al. 2016).

In a crossover overnight study made by Sandberg et al., 21 healthy subjects were given 4 rye-based evening test meals. The test meals consisted of whole grain rye flour bread (RFB) or whole grain rye flour and rye kernels bread (RFB/RKB) in a 1:1 ratio, with or without added resistant starch (+RS). White wheat flour bread (WWB) was used as reference meal. The RFB/RKB + RS combination positively influenced biomarkers of glucose by significantly decreasing postprandial glucose and insulin response (p <0.05). It also increased the serum PYY gut hormone on the following morning. RFB and RFB/RKB improved subjective appetite ratings. Alone or combined with rye kernels, RFB increased the sensation of satiety and lowered perceived hunger sensations at 11–14.5 h after intake (Sandberg et al. 2017).

In women with diabetes, whole grain and bran intakes were associated with reduced all-cause and CVD-specific mortality (He et al. 2010). Consumption of whole grain breakfast cereal and bran was significantly associated with a lower incidence of ischemic stroke (Consumption 2018).

Arabinoxylan (AX) is a hemicellulose synthesized by glycosyltransferases (membrane enzymes): arabinoxylan synthase, transferase, and feruloyl transferase (substitutes ferulate into arabinose residues) (Mitchell et al. 2007). AX is abundant in cereal endosperm cell walls of rye and wheat, followed by oats, barley, maize, and rice. Arabinoxylans display functional properties including viscosity enhancement,

gel formation, emulsion or foam stabilizations, water absorption, fat replacement, and prebiotic activity. As constituents of dietary fiber, arabinoxylans also have nutritional and health-promoting benefits.

Arabinoxylan may help manage diabetes, according to a small study published in the *European Journal of Clinical Nutrition* in 2004. Fifteen patients with T2DM supplemented their diets with arabinoxylan-enriched bread and muffins or with bread and muffins made with white wheat flour for 5 weeks. The arabinoxylan-enriched bread group showed significant improvement in blood sugar control. Arabinoxylan may also help regulate blood sugar in people without diabetes, as a small study published in the *American Journal of Clinical Nutrition* suggests. After consuming a breakfast containing arabinoxylan-enriched bread, 14 healthy participants were found to have significantly lower blood sugar compared to consumption of normal bread.

30.5.2 Gastrointestinal Disease

30.5.2.1 Regulation of Microbiota

The human intestinal tract is populated with a great variety of bacteria, the microbial ecosystem (gut microbiota) with an essential role in maintaining functionality and homeostasis. Western diet rich in processed and fiber-poor foods and the use of antibiotics alter the composition of gut microbiota and lead to dysbiosis (Wypych and Marsland 2017). Dysbiosis is associated with gastrointestinal disease, allergy, T2DM, and intestinal inflammation (Hiippala et al. 2018). The gut microbiota is populated with *Bifidobacterium*, *Bacteroides*, *Proteobacteria*, *Firmicutes*, *Acinetobacter*, and *Clostridium*. The composition of microbiota can vary depending on clinical conditions like inflammatory bowel disease and nonalcoholic fatty liver disease (Takahiro et al. 2017). The role of gut microbiota in these diseases occurs through proinflammatory signaling and via direct hepatotoxicity of bacterial products (Parnell et al. 2012).

Different studies have shown a strong correlation between gut microbiota composition and dietary patterns. For example, the gut microbiota of people who consume a high-fiber diet is dominated by *Bacteroidetes* (Takahiro et al. 2017; El Khoury et al. 2012). Dietary fiber can modulate composition and metabolic function of human microbiota. Dietary patterns have a strong impact in bacterial diversity and production of microbial-derived fecal fermentative end products. Cross-sectional studies in human populations revealed that fiber-rich diet is associated with increased gastrointestinal microbial community diversity. In Europe the consumption of dietary fiber is on average 16–29 g/day and in the United States 12–18 g/day. A consumption of 10 g/day dietary fiber can increase the abundance of *Bifidobacterium* and *Lactobacillus* species in gastrointestinal microbiota and stimulates changes in *Bacteroidetes* and *Firmicutes* bacterial species (Holscher 2017).

In a study comparing 15 healthy European children consuming a high-protein and animal fat diet with 14 healthy African children on a high-fiber diet, de Filippo et al.

detected differences in the fecal microbiota. In European children, the microbiota was dominated by *Firmicutes*, and in Africans it was dominated by *Bacteroidetes* (De Filippo et al. 2010).

A cross-sectional study on 28 healthy Japanese women also revealed a relationship between dietary patterns and the fecal microbiota. The fecal microbiota was analyzed by the terminal restriction fragment length polymorphism (T-RFLP) method. T-RFLP pattern analysis divided the subject in two clusters: cluster A (abundance of *Bacteroidetes* and *Clostridium*) and cluster B (abundance of *Bifidobacterium* and *Lactobacillales*). Cluster A included 12 subjects with low-protein diet, and cluster B group included 16 subjects with fiber-rich diet, especially mushrooms rich in prebiotics like inulin, β-glucan, and fructooligosaccharides. These prebiotics increased the number of beneficial bacteria, like *Bifidobacterium* and *Lactobacillales*. Bifidobacteria have an important role in intestinal health and a healthy gut microbiota (Takahiro et al. 2017).

In a recent study on 28 subjects, Eslamparast et al. demonstrated that prebiotic fibers have beneficial effects in the composition and activity of gut microbiome. Prebiotics stimulate bacterial production of short-chain fatty acids, reduce the luminal pH, inhibit the growth of pathogens, and improve glucose and lipid metabolism (Eslamparast et al. 2017).

30.5.2.2 Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD), the most common cause of chronic liver disease, is considered the hepatic manifestation of the metabolic syndrome (Shen et al. 2017).

In a single-center double-blind, placebo-controlled, parallel-group study on 30 participants with NAFLD, the efficacy of prebiotic supplementation in reducing liver fat and hepatic injury was demonstrated. The patients were randomized to a 16 g/day prebiotic-supplemented group and an isocaloric placebo group for 24 weeks. The liver enzymes (ALT, AST), TNF-α, and total cholesterol were reduced in the group with prebiotic supplementation demonstrating improvement of liver injury. The authors consider that prebiotics can modify the gut barrier integrity and endotoxin translocation, as well as increase *Bifidobacterium* with beneficial role in NAFLD (Lambert et al. 2015).

A study on 32 patients with NAFLD for 6 months evaluated the association between fiber intake and zonulin (ZO) concentration as marker of intestinal permeability. During the study the dietary fibers were increased from 19 g/day to 29 g/day: soluble fiber (6.69 ± 3.21 g per day) and insoluble fiber (17.44 ± 7.11 g per day). This study demonstrated a significant improvement of liver injury markers serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGTP), decrease of ZO concentration, and improvement in hepatic steatosis (Krawczyk et al. 2018).

A recent study demonstrated that a higher insoluble fruit fiber consumption (≥7.5 g/day) was associated with improved liver health and improvements in fatty liver parameters. The authors speculate that the production of short-chain fatty acids

from fermented fibers ensures the preservation of the intestinal barrier and has a protective and nourishing role for colonocytes (Cantero et al. 2017).

Another study on 31 subjects demonstrated that a Mediterranean diet associated with monounsaturated fatty acids and prebiotics reduced hepatic fat and improved insulin sensitivity even without weight loss in NAFLD patients (Abenavoli et al. 2014).

30.5.2.3 Inflammatory Bowel Disease

Inflammatory bowel diseases represent disorders of the gastrointestinal tract characterized by immune activation and consequently chronic relapsing inflammation that include Crohn's disease and ulcerative colitis.

A recent study demonstrated the effectiveness of a mixture of digestive enzymes, beta-glucan, and inositol in inflammatory bowel disease. The study included 43 participants randomized in 2 groups: group A ($n = 23$) with conventional treatment (topical and systemic mesalamine) plus one tablet after lunch and dinner of a mixture of digestive enzymes, beta-glucan, and inositol for 4 consecutive weeks and group B ($n = 20$) which was given only conventional treatment. The mixture contributed to improved quality of life and reduced abdominal pain, bloating, and flatulence in patients with inflammatory bowel disease, improving their overall clinical condition. β-Glucan had a prebiotic effect and reduced the level of pro-inflammatory cytokines (IL10, IL12, and TNF-alpha). The authors demonstrated that inositol reestablished intestinal motility and improved gastrointestinal symptoms, also restoring intracellular calcium levels (Spagnuolo et al. 2017).

30.5.2.4 Colonic Cancer

Lifestyle (physical inactivity) and dietary patterns (poor diet) play a significant role in the development of colorectal cancer. It is the third most commonly diagnosed cancer in men and the second most commonly diagnosed in women worldwide. The symptoms of colorectal cancer are bloody stools, fatigue, and bowel movement changes (constipation, diarrhea, or narrowing of the stool). The risk of colorectal cancer is significantly increased by inadequate fiber intake and inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis (Emilsson et al. 2017). Dietary patterns are useful for the investigation of chronic disease development. The term dietary pattern refers to several techniques of defining total diet quality (Brown and Fung 2013). Current dietary recommendations to prevent colorectal cancer include eating a variety of vegetables, fruits, and whole grains rich in fiber while limiting consumption of processed foods and less red and processed meats (Perera et al. 2012). Recent studies demonstrate that diets high in fiber can be preventive against colorectal cancer, as every 10 g of fiber consumed daily reduces risk by 10% (Aune et al. 2011).

A recent randomized controlled trial entitled BENEFIT (**B**eans/**B**ran **E**nriching **N**utritional **E**ating **F**or **I**ntestinal health **T**rial) determined the feasibility of increasing dietary fiber intake in colorectal cancer survivors and examined serum inflammatory biomarkers. There were 29 participants who successfully completed the 4-week pilot dietary intervention trial, consuming 35 g/day navy bean powder or

30 g/day rice bran that allowed them to meet the 5% dietary intake level shown to inhibit colon tumor formation. This pilot study established that a fiber-rich diet in colorectal cancer survivors has a chemopreventive effect, maintains a healthy gut through decreased oro-fecal transit time, produces short-chain fatty acids that reduce gut inflammation, and promotes apoptosis (Borresen et al. 2016).

Rice bran is rich in dietary fibers, γ-oryzanol, tocotrienols, β-sitosterol, and essential vitamin E complex. Rice bran extracts have been reported to contain biologically active compounds with chemopreventive, anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic properties. In human colorectal cancer, they inhibit cell proliferation and angiogenesis, induce apoptosis, and stimulate the immune system. A recent study demonstrated that purple rice bran oil has a chemopreventive role in colorectal cancer, significantly suppressing inflammation and decreasing the production of interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and leukotriene B4 (LTB4) (Sirithunyalug et al. 2018).

The immunomodulating effects of arabinoxylan bran MGN-3 were examined by Ghoneum in 32 cancer patients with various malignancies including colon cancer, with depressed NK cell activity after conventional cancer treatment. Treatment with MGN-3 for 2 weeks led to a significant tenfold increase in NK cell activity, increase in NK cell binding capacity, and granularity and improvement in tumor-associated antigens and in T and B cell proliferation in vivo in a selected number of patients (Ghoneum and Gollapudi 2003).

30.5.3 Obesity and Weight Reduction

The prevalence of obesity is increased in developed and developing countries throughout the world. Obesity is associated with an increased risk of diseases, like cardiovascular disease, hypertension, dyslipidemia, and different forms of cancer. Weight loss has lots of health benefits, for example, it improves insulin sensitivity and decreases adipose tissue inflammation (Strączkowski et al. 2018). The consumption of products rich in β-glucan is associated with weight reduction, because they can reduce energy intake and are able to modulate appetite. β-Glucans have the ability to form highly viscous solutions and have the property of fermentability, which brings health benefits and contributes to weight reduction. It was demonstrated that diets rich in fibers have a positive contribution to long-term weight management, reduce LDL cholesterol, and improve glycemic control (El Khoury et al. 2012).

The soluble dietary fiber glucomannan, with a strong water-holding capacity, reduced energy intake, increased satiety, and produced a significant reduction of weight at a dose of 1.24 g daily in a study on 1076 subjects with an energy-restricted diet. The duration of the study was 5 weeks (Birketvedt et al. 2005).

In a critical review, it was concluded that soluble fibers with viscosity-producing properties, such as guar gum, pectin, psyllium, and β-glucan, are more strongly associated with reduced hunger and/or appetite perceptions than low-fiber diets (Dikeman and Fahey 2006).

In a large European study with a 6.5 year follow-up, consumption of 10 g/day total dietary fiber was associated with significant weight reduction and waist circumference reduction (Du et al. 2010).

In a recent review, Bozzetto et al. revealed the beneficial effects of dietary fiber on blood glucose metabolism, LDL cholesterol, blood pressure, and plasma triglycerides (Bozzetto et al. 2018).

30.6 Applications in Foods

In recent years various fiber ingredients have been added to formulated foods to increase fiber intake. A healthy dietary pattern includes whole foods rich in fibers from vegetables, whole grains, fruits, nuts, and seeds, in order to achieve the daily recommended amount for dietary fiber and also fulfil the needs for other important nutrients. Adding fiber to food can provide an alternative way to reach recommended intake levels. Functional fibers derived from wheat, corn, and rice have been commonly accepted in various food applications, both for their health attributes and technical functionalities. A recent trend in food industry explores the utilization of novel or uncommon sources of dietary fiber from processing of fruits, vegetables, and seeds. Dietary fiber extracts and isolates are obtained from different sources based on certain preparation methods and have various physicochemical properties, leading to many applications in food product development. From a functionality perspective, any fiber can play a number of roles. For example, citrus fiber may be used as bulking agent in reduced-sugar products, to improve texture, to manage moisture, and to add color, and as natural antioxidant (Viuda-Martos et al. 2010b).

Dietary fibers display a plethora of activities when they are incorporated in food systems, due to their water-binding capacity, gel-forming ability, and fat mimetic properties. Fibers contribute to improvement of texture, sensory characteristics, and shelf life of foods through antisticking, anticlumping, texturizing, and thickening effects (Yangilar 2013). For example, in bakery products, fibers prolong freshness; in bread they can modify softness, loaf volume, and firmness. In meat products they improve texture, fat binding, cooking yield, and water binding (Yangilar 2013).

Drinks and beverages containing soluble fibers (pectins, fractions of grains, and multifruits, polidextrose, ß-glucans, cellulose beetroot fiber) are the most used. Added soluble fibers increase their viscosity and stability (Yangilar 2013). Bakery products and breakfast cereals are enriched with dietary fibers derived from fruits because of their nutritional quality, strong antioxidant capacity, high amounts of total and soluble fiber, less caloric content, and greater grade of fermentability and water retention. In dairy products the most used fiber is inulin, which reduces syneresis in yoghurt and fermented milk products and improves palatability in cheeses and ice creams. Pectins are used for elaboration of marmalades and jams, because they stabilize the final product. In low-calorie products, sugar is substituted by inulin and oligofructose.

Recently, enzymatic treatments have been reported to alter fiber qualities and increase their solubility and functional properties. Mrabet et al. used Viscozyme® L

for the first time in 2016 to convert insoluble fiber in dates to soluble fiber, leading to a functional ingredient with increased antioxidant activity and higher concentrations of prebiotic oligosaccharides (Mrabet et al. 2016). In 2017 Wen et al. studied the effects of enzymatic treatment in combination with micronization on structural and functional properties of rice bran, resulting a fine fiber powder with increased soluble-to-insoluble fiber ratio, reduced water and oil holding capacity, increased swelling capacity, and improved absorption capacity for cholesterol and sodium taurocholate (Wen et al. 2017).

30.7 Safety: Toxicity and Side Effects

Although a recommended dietary allowance, or RDA, has not been set for fiber because of insufficient evidence, the Food and Nutrition Board has set an Adequate Intake for the nutrient. It is the amount that should satisfy the needs of most healthy individuals within a specified age range. While fiber has a number of health benefits for the body, eating too much can lead to uncomfortable side effects and potentially serious complications.

Even though fiber has numerous benefits, too much of it can be harmful. Since fiber is not actually digested by the body, the digestive tract has a more difficult task moving it, which may cause cramping, bloating, gas, and abdominal discomfort. Not drinking enough water to help move the bulk leads to constipation. If the constipation isn't resolved, it can lead to an intestinal blockage, the most dangerous side effect associated with excessive fiber intake. An intestinal blockage can lead to nutrient malabsorption and a dangerous accumulation of toxins in the body.

The 2009 Codex Alimentarius Commission definition of dietary fibers dividing them into three categories introduced items obtained from food raw material and synthetic carbohydrate polymers (Codex Alimentarius Commission 2010). These have to provide physiological effects comparable to natural fibers occurring in foods. The Codex recommends consumption of 14 g fiber/1000 kcal, for both genders at all age groups. Although no tolerable upper intake level has been set for dietary fiber, it is recommended that women eat 25 g of fiber a day and men 38 g per day. Above 70 g could lead to negative effects, leading to reductions in the absorption of some minerals. It is not clear yet if this could create mineral deficiencies.

Psyllium seed is not recommended to patients with fecal impaction and undiagnosed abdominal pain or other symptoms, a sudden change in bowel habit longer than 2 weeks, nausea, vomiting, rectal bleeding, and constipation. It is also not to be used by patients with diseases of the esophagus and cardia, ileus, or megacolon. Psyllium seed is not to be used by patients with known hypersensitivity to psyllium or any of the other constituents of the product.

Green bananas are rich in resistant starches, which can increase the risk of constipation. In underripe bananas starch constitutes 80–90% of the carbohydrate content, which as the banana ripens changes into free sugars (Hermansen et al. 1992). They also contain high doses of tannic acid which has an inhibitory effect on

the digestive tract and gastrointestinal motility and obstructs secretion of gastrointestinal fluids (Samanta et al. 2004).

Safety data on long-term or regular use of arabinoxylan are currently lacking. However, since arabinoxylan may lower blood sugar levels, there's some concern that using arabinoxylan in combination with diabetes medications may have harmful effects.

Supplements created as combinations of fiber and herbs are not always tested for safety and might also be unregulated. They may contain doses that differ from the labeled amount for each herb, or they may be contaminated with heavy metals. The safety of such supplements in children, pregnant women, nursing mothers, and patients on different medications is not known, and unexpected herb-drug interactions might occur.

30.8 Marketed Products

Food developers face technical challenges during formulation design of new products, as single formulations cannot easily achieve the high level of recommended daily intake of dietary fiber. The impact of dietary fiber materials can be unpredictable for sensory properties and product quality. Another problem can be the massive production using automated assembly lines which can affect integrity, palatability of fiber-fortified foods, and bioavailability (Table 2).

Table 2 The properties of dietary fiber: material science-oriented physicochemical properties, nutrition-oriented physiological effects, and food application-oriented technological functionalities (Kendall et al. 2010; Rastall 2010; Viuda-Martos 2010b; Brownlee 2011)

Physicochemical properties (material physics/chemistry)	Physiological effects (biomedical and nutritional area)	Technological functionality (food product development)
Solubility	Changes in intestinal function	Water binding/holding capacity
Viscosity	Reduction of cholesterolemia	Fat/oil binding and retention capacity
Density and bulk volume	Modification of the glycemic response	Viscosity and rheological property
Surface area characteristics and porosity	Laxation Satiety	Gel-forming capacity and swelling
Particle size	Fermentation in the colon	Fermentative capacity
Cation exchange capability	Reduction of nutrient availability	Metal ion-chelating capacity
Chemical reactivity/interaction with other organic molecules including fat/oil, protein, vitamins, antioxidants	Enhanced health benefit through synergistic effects with other active ingredients	Texturizing capacity – thickening, bulking, texture modification Flavor modification Control of sugar crystallization

Table 3 Trends of dietary fiber utilization in novel food product development (van der Kamp et al. 2010)

Approaches	Food applications
Use of fiber-rich raw materials containing intrinsic and intact fiber for conventional food production	Bread, breakfast cereals, pasta and noodles, bakery goods, extruded snacks
Addition of isolated, purified fiber in nontraditional DF carriers for enrichment or fortification	Meat, fishery, and dairy products including fermented beverages
Utilization of processing waste stream or by-product rich in fiber for formulation improvement	Restructured meat and fishery products, bread, extruded snacks

The food industry incorporates dietary fibers into a wide variety of foods, such as pasta, breads, baked products, breakfast cereals, yogurts, vegetable milk, and fermented beverage (Table 3). The acceptable level of fiber incorporation to bakery and pasta formulations is limited to 5%, while the addition above 10% yields some unfavorable alterations to product quality characteristics such as color and texture and determines loss of cooking and sensory properties (Li and Komarek 2017).

Adding fibers to foods from a variety of sources promotes positive health benefits and has been successful in reducing nutrient deficiencies. According to current regulations, a food item can carry the claim of "more or added fiber" when it contains at least 2.5 g of fiber or more per serving than the reference food. A "good source of fiber" claim can be made when the food contains 2.5 to 4.9 g of fiber, and a "high fiber" claim can be made when a food item contains 5 g or more fiber per serving (Hazen 2012).

Dietary fibers are used in various formulations like flours, premixes for breads and baked products, breakfast cereals, pasta, fortified milk, fortified yogurts, fortified beverages, fortified juices, granola and cereal bars, and fortified plant-based milk and yogurts.

Dietary fiber supplements are also marketed in various forms like pills, capsules, powders, and gels. Dietary fiber supplements can contain one or more dietary ingredients, minerals, vitamins, or herbs.

Pure psyllium powder, a one-ingredient supplement that supports satiety and offers digestive benefits delivers the large amount of 4.5 g of fiber per teaspoon, which immediately begins to gel once mixed with water. Another product is an orange-flavored powder that looks like fruit punch mix smoothie once added with water, being surprisingly tasty. Psyllium powder capsules are designed without side effects like gas and bloating, being made of calcium polycarbophil to help regulate bowel movements and eliminate diarrhea and constipation. Two capsules should be taken with eight glasses of fluid. Another sugar- and gluten-free supplement contains 6 g of psyllium fiber and is recommended up to three times per day.

A vegan gluten- and dairy-free formula contains 9 g of fiber, 9 g of protein, and 1 g of sugar sweetened with stevia. It is made from 15 raw organic superfoods, including sprouted seeds and legumes, grains, probiotics, and omega-3 fatty acids, in 120-calorie servings.

Fibersol® is a line of fiber ingredients developed for homemade baking products for health-conscious consumers with many functional benefits, like replacing calories from sugar and fat and increasing satiety.

Inulin is an excellent dietary fiber with significant health-promoting benefits. It is used in many applications, including bakery, cereals and cereal bars, dairy, infant nutrition, beverages, confectionery, ice cream, and healthcare nutrition. Inulin can be used as sugar, as a texturizer, and as fat replacer. In infant formulas it improves the intestinal microflora of babies and small children, mimicking the prebiotic effect of mother's milk. Inulin's application in confectionery highlights its sugar-replacement properties, resulting in low-calorie products. Optionally inulin can be used in a high-quality, sugar-free chocolate or "low-fat," "low-sugar," or "low-calorie" ice creams, sorbets, or frozen desserts with good texture and taste. Inulin can enrich the fiber content of soups, sauces, and seasonings. Noodles are a popular food, particularly in Asia, and inulin supplementation provides an excellent opportunity to promote a healthier lifestyle by increasing the intake of dietary fiber and helping control body weight.

Glucomannan, a water-soluble super fiber supplement derived from the elephant yam, has shown promise as an effective weight-loss supplement. It will absorb 50 times its own weight in water, so when taken before a meal with a glass of water, it creates a full feeling in the stomach.

30.9 Patents

At present, dietary fibers have various applications and patents in food development.

The patent of Aldritt et al. refers to an effervescent formula that included water-soluble dietary fiber for laxative effects, which also decreased cholesterol; increased the absorption of vitamins and minerals, especially dietary calcium; and promoted the growth of beneficial bifidobacteria in the lower gut. The composition included at least 2.5 g dietary fiber, 50–1250 mg sorbitol, 5–500 mg sodium benzoate, and 5–500 mg polyethylene glycol. The effervescent formulation had lots of benefits: can be a useful dosage form for delivering active agents, can be dissolved rapidly, was easier to swallow, and could be packaged in controlled quantities (Aldritt et al. 2006).

Another patent created a low molecular weight dietary fiber material, obtained by using enzymes which hydrolyzed any starch into small molecules and only partially hydrolyzed β-glucan molecules. The advantages of this dietary fiber material with β-glucan were low molecular weight with excellent physicochemical, physiological, and sensory properties and a particular polydispersity, low viscosity, and high stability in water. This dietary fiber material had neutral palatability and could be used in baked goods, bars, ice cream, yogurt, beverages, or other foods without affecting the sensory attributes of the food. The modified β-glucan with an average molecular weight ranging from 50 to 1000 kDa had a therapeutic role in regulation of glycemic response, reduction of cholesterol, enhancing the growth of bifidobacteria

and immune activity, and lowering the risk of cardiovascular and intestinal diseases (Milan and Peal 2013).

A patent created as dietary fiber gel filling reduced the caloric and fat content of prepared sandwiches with minimal effect on texture and taste. This dietary fiber gel filling replaced a portion of fat of prepared sandwiches by the content of β-glucan from yeast, soluble fibers derived from flax seed, vegetable, and fruit fiber sources. The concentration range for β-glucan was from 5% to 15% of the composition. The advantage of the present invention was a higher fiber alternative to traditional prepared sandwich type foods that reduced caloric and fat content. This dietary fiber gel can be used not only for sandwiches but also for tacos, crepes, pies, pancakes, and wraps (Evans et al. 2014).

An invention of a formulation of soluble, functional fiber ideal for addition to liquid foods and nutritional formulas included fructooligosaccharides (FOS) and partially hydrolyzed guar gum (PHGG). PHGG is entirely fermented in the colon and increases the production of butyrate that plays an important role in the regulation of cell differentiation, proliferation, and apoptosis in the intestine. PHGG 21 g/ day is considered a prebiotic and increases the concentration of the beneficial bacterial strains lactobacilli and bifidobacteria. Fifteen grams per day PHGG reduces constipation. FOS are short-chain fructose polymers with prebiotic activity that also stimulate the growth of lactobacilli and bifidobacteria. FOS 4 g/day suppresses growth of pathogenic bacteria and enhances a positive immune response. This patent provides a dietary fiber formulation with great prebiotic potential (Ca and Street 2008).

The process of making a flour composition with resistant starch for increased total dietary fiber was the reason behind several other patents. These fortified flours have numerous beneficial effects: reduced caloric value, reduced glycemic response, controlled glucose regulation, impacted satiety, sustained energy release, improved athletic performance, and mental concentration and memory. High-amylose flours are processed with a short hydrothermal treatment to increase total dietary fiber content. The native source can be corn, tapioca, rice, wheat, sweet potato, oat, amaranth, pea, sorghum, and barley. The high-amylose flours contain 27% amylose for wheat or rice and 40% amylose for other sources. These flours can be used for breakfast or expanded cereals, breads, cakes, muffins, pastry, pasta, snacks, fried and coated foods, yogurts, cheeses, sour creams, and beverages (Evans et al. 2014).

The invention of a pouch with pores was intended to deliver the daily recommended soluble fiber intake, having one portion placed into the mouth to release the quantity of soluble dietary fibers desired. The pouch can contain flavorants, sweeteners, minerals, vitamins, energizing agents, and antimicrobial agents (Schleef et al. 2008).

30.10 Future Perspectives

The simplified definition of dietary fiber as a carbohydrate that resists digestion and absorption and that may or not undergo microbial fermentation in the large intestine is essential for correlating consumption levels and health benefits. Dietary fibers

have many different constituents, of particular interest being inulin, β-glucan, pectin, bran, arabinoxylan, and resistant starches, which all play an important role in human health. Although further research is needed to better understand particular health claims and mechanisms involved, current research is oriented toward all these constituents.

An inverse relationship has been reported between fiber consumption and the risk for coronary heart disease and various types of cancer. The FDA has launched the claim that increased intake of dietary fiber can reduce the incidence of coronary heart diseases and cancer and although not yet adopted, dietary fiber is suggested to have a role in obesity and diabetes. The mechanisms supporting these claims are still unclear; some data are contradictory, but the majority of studies agree with the fact that the lower energy density of dietary fiber could decrease the gross energy of a food.

Much of the recent literature has been focusing at the ability of different dietary fiber types to affect certain physiological systems, especially trying to understand the influences on fermentation patterns of the intestinal microbiome. The prebiotic function of fibers is intensely studied using new DNA sequencing procedures that will permit to completely characterize the bacterial populations involved in the future. Butyrate as product of fermentation regulates gene transcription acting as a histone deacetylase inhibitor, which influences cell proliferation, differentiation, and apoptosis of colonocytes. The goal of these studies will be to determine individual differences and risk to develop diseases and consequently personalize dietary modifications that improve human health.

In a recent review on evidence base of BioBran/MGN-3 arabinoxylan compound as a complementary therapy for conventional cancer treatment, Soo Liang Ooi et al. examine the current evidence from 32 preclinical studies, clinical case reports, and small clinical trials that confirm the upregulating of the patient's immune system, especially by boosting NK cell activity. MGN-3 is considered safe with no adverse event reported. The combination of MGN-3 with new biological targeting treatments may open future perspectives in tumor therapy. The authors consider that additional pharmacokinetic studies and well-designed RCTs to strengthen the evidence for clinical application are needed (Ooi et al. 2018).

The main interest for further studies in specific areas of dietary fiber research includes the components of fibers such as β-glucan, arabinoxylan, and resistant starches.

30.11 Cross-References

- ▶ Oligosaccharides in Food
- ▶ Polysaccharides in Food
- ▶ Starch

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Oligosaccharides in Food

31

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Contents

Abstract

Oligosaccharides are small molecular carbohydrates comprising of 3–10 monosaccharides. They have shown beneficial effects on immune system and gut health, such as anti-allergic, anti-inflammatory, antidiabetic, anticancer, antiaging, and anti-obesity activities. Functional oligosaccharides are commonly found in plants, algae, bacteria, and higher fungi. Milk is perfect for human

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health improvement. The biological functions of milk oligosaccharides, especially human milk oligosaccharides, are known to have considerable health benefits for humans, such as the growth promotion to the beneficial intestinal flora and the resistance to the infections of bacteria and virus. Oligosaccharides synthesis is becoming increasingly important to pharmaceutical industry, in which chemoenzymatic syntheses are considered as effective ways. The functional oligosaccharides have been widely applied in various branches such as pharmacological supplements and food ingredients. This chapter gives a brief summary about the chemical characteristics, the potential health benefits, the application in foods, and marketed products of the functional oligosaccharides.

Keywords

Oligosaccharides · Bioactive · Gut health · Human milk oligosaccharides · Application

31.1 Introduction

Before the 1960s, sugars were just generally regarded as the chemical energy and structural substances. With the development of molecular biology, medicine sciences and other disciplines in recent years, people gradually realized that carbohydrates perform numerous roles in living organisms except for the storage of energy (e.g., starch and glycogen) and as structural components. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development. According to the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature, naturally occurring carbohydrates that consist of 3–10 monosaccharide units, linear or branched, connected by α- and/or β-glycosidic linkages, are defined as oligosaccharides (Zhao et al. 2017). Plants and algae are the richest sources of functional oligosaccharides (Zhao et al. 2017). Recently, much attention has been paid by consumers on natural bioactive compounds to be used as functional ingredients in nutraceuticals. Functional oligosaccharides are intermediate between monosaccharide and polysaccharides and are well known as prebiotics, due to they benefit the host by selectively stimulating beneficial bacteria in the intestine, especially *Bifidobacterium* species (Xu et al. 2009). Compared with ordinary oligosaccharides, functional oligosaccharides are zero or low calorie and have more unique physiological functions, such as promoting *Bifidobacterium* proliferation, improving the body's immunity, preventing dental caries and constipation, and inhibiting tumor, etc. Moreover, functional oligosaccharides have a positive effect on different well-being aspects of the host, such as immune modulation, intestinal health, gastric microbial function, calcium absorption, and bone mineral density, particularly among adolescents (Kumar et al. 2016). The types of oligosaccharides, known as

research focus, are marine oligosaccharides, fructo-oligosaccharides (FOS), chitosan-oligosaccharides (COS), human milk oligosaccharides (HMOs), galacto-oligosaccharides (GOS), etc. The FOS existed in fruits and vegetables such as onions and bananas are difficult to be eliminated and absorbed by human body. However, it can be easily used by *Bifidobacterium* as a bioactive factor for proliferation. Marine oligosaccharides which mainly contained agar oligosaccharides and carrageenan have huge development potential and have attracted broad attention in drug discovery (Yang et al. 2019; Zhao et al. 2015, 2018). Marine algae are rich in sulfated polysaccharides (SPs) such as carrageenans in red algae, fucoidins in brown algae, and ulvans in green algae (Ngo and Kim 2013) and considered as valuable sources of structurally diverse bioactive compounds. COS as a biocompatible compound is derived from the hydrolysis and the deacetylation of chitin, which is one polymer of N-acetyl-D-glucosamine and abundantly found in the exoskeleton of arthropods including crabs and shrimps (Mattaveewong et al. 2016). The concentration of HMOs in mature human milk is 10–15 g/L, which is 100- to 1000-fold higher than that in bovine milk, and its content often exceeds the total amount of protein in human milk (Zhao et al. 2017). GOS was produced in the course of lactose hydrolysis and due to the transgalactosydic reaction by the enzyme β-galactosidase produced (Fischer and Kleinschmidt 2018). GOS can reach the large intestine and fermented by the gut microbiota, and it also can exert various health benefits for the host by improving the growth of probiotic bacteria (Panesar et al. 2018). Functional oligosaccharides were found widely in plants, algae, bacteria, and higher fungi. They have become the research hotspots because of which cannot be digested in the gastrointestinal tract (Pan et al. 2018a). Oligosaccharides have large amounts of important biological activities, application, and development in health food engineering. And functional oligosaccharides continue to stimulate great interest and have potential future value. So, the different uses of oligosaccharides have been studied in structural chemistry, molecular biology, medicine, and food science fields.

31.2 Bioactive Constituents

Oligosaccharides exhibit a high degree of diversity in structure. In contrast to polysaccharides, the structure of oligosaccharides has been well analyzed. This numerous structure diversities lead to highly specialized and selective interactions that play key roles in their activities. In general, oligosaccharides are usually composed of five different monosaccharides: glucose, galactose, *N*-acetylglucosamine, fucose, and sialic acid (Morozov et al. 2018). The known functional oligosaccharides have included arabino-oligosaccharides, arabinogalacto-oligosaccharides, arabinoxylo-oligosaccharides, galacturono-oligosaccharides, rhamnogalacturonoligosaccharides, and HMOs (Table 1). These natural oligosaccharides can improve the balance and activity of the intestinal microflora and greatly prevent the gastrointestinal infections (Fig. 1). Oligosaccharide chains (glycans) are linked to lipids or to compatible amino acid side chains

Table 1 Natural functional oligosaccharides and their glycosidic linkages

Types	Monosaccharides	Number of monosaccharides	Bonds indicative of functions
Arabino-oligosaccharides	Arabinose	2–8	α-1,5
Arabinogalacto-oligosaccharides	Arabinose, galactose	2–9	β-1,4
Arabinoxylo-oligosaccharides	Xylose, arabinose	5–10	α-1,2, α-1,3, β-1,4
Clycosylsucrose	Glucose, fructose	3	α-1,2, β-1,4
Cyclodextrins (CDs)	D-glucopyranose	6 (α-CD), 7 (β-CD), 8 (γ-CD)	α-1,4
Fructo-oligosaccharides	Sucrose, fructose	2–5	β-1,2
Galacto-oligosaccharides	Galactose	2–5	β-1,2, α-1,4
Galacturono-oligosaccharides	Galactosamine	2–9	α-1,4
Gentio-oligosaccharides	Glucose	2–10	β-1,6
Glucose-oligosaccharides	Glucose	2–10	α-1,2, β-1,3, β-1,6
Human milk oligosaccharides	Glucose, galactose, GlcNAc	2–8	α-1,2, α-1,3, α-1,4, α-2,3, β-2,6, β-1,3, β-1,4
Isomalto-oligosaccharides	Glucose	2–5	α-1,4
Lactosucrose	Galactose, fructose	2–3	β-1,4
Lactulose	Galactose, fructose	2	β-1,4
Malto-oligosaccharides	Mannitose, glucose	2–10	α-1,2, α-1,4
Palatinose	Glucose, fructose	2	β-1,6
Raffinose	Galactose, fructose, glucose	3	β-1,2, α-1,4
Rhamnogalacturonoligosaccharides	Rhamnose, galactose	4–8	α-1,2, α-1,4, β-1,4
Soybean oligosaccharides	Fructose, galactose, glucose	2–4	α-1,6
Stachyose	Galactose, fructose, glucose	4	α-1,4
Xylo-oligosaccharides	Xylose	2–7	α-1,4

in proteins by *N*- or *O*-glycosidic bonds (Fig. 1). *N*-linked glycosylation almost always involves oligosaccharide attachment to asparagine via β-linkage to the amine nitrogen of the side chain (Angelyn and Barbara 2011). And then, oligosaccharides that participate in *O*-linked glycosylation are attached to threonine or serine on the hydroxyl group of the side chain (Madson 2016). Modification of the glycosylation has enriched the biological activities of oligosaccharides.

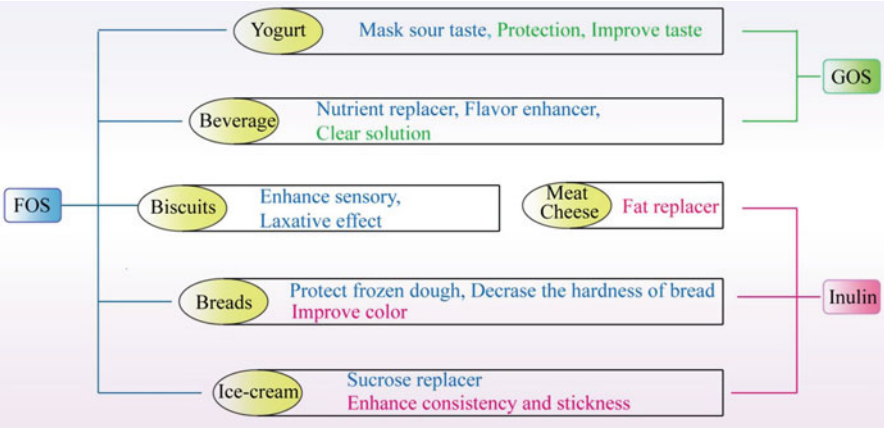

Fig. 1 The effects of GOS, FOS, and inulin on food

31.3 Bioactivities

31.3.1 Anti-allergic Activity

Allergic diseases are one of the public health problems over the world. It is estimated that about one-third of the general population and one-fifth of the population in western countries were troubled in allergic diseases. In essence, allergy is caused by an exaggerated adaptive immune response to harmless environmental substances, such as animal dander, house dust mites, foods, pollen, insects, and chemical agents (Ngo and Kim 2013). Many researchers have made a series of experiments against allergy. Maternal dietary supplemented with specific nondigestible oligosaccharides during pregnancy in mice led to reduce allergic asthma symptoms in the offspring (Hogenkamp et al. 2015). Further experiment showed that the dietary intervented with nondigestible oligosaccharides and partial hydrolyzed whey protein prevented the onset of food allergic symptoms in mice (van Esch et al. 2017). In recent years, COS with low molecular weight has been proven to exhibit protective effect against ovalbumin-induced lung inflammation in a mouse model of asthma. Oral administration of low-molecular-weight COS (LM-COS) (16 mg/kg body weight/day) has resulted in a significant reduction in both mRNA and protein levels of interleukin (IL)-4, IL-5, IL-13, as well as tumor necrosis factor (TNF)-α in the lung tissue and bronchoalveolar lavage fluid (BALF). Moreover, compared with the ovalbumin-sensitized/ovalbumin-challenged asthma control group, the protein levels of IL-4, IL-13, and TNF-α in BALF were decreased by 5.8-, 3.0-, and 9.9-fold, respectively. These results suggested that the oral administration of low-molecular-weight COS was effective in alleviating the allergic inflammation in vivo. And it could be an excellent source for the development of a potent therapeutic agent against mast cell-mediated allergic inflammatory responses and airway inflammation in allergic

inflammatory diseases, including asthma (Chung et al. 2012). *Eucheuma cottonii* sulfated oligosaccharide (ESO) that shows anti-food allergy activity in RBL-2H3 cells and BALB/c mouse model in vivo possesses preventive and therapeutic potential in allergic disease (Xu et al. 2017). ESO has decreased the levels of mast cell protease-1 and histamine, as well as inhibited the level of specific immunoglobulin (Ig) E up by 77.7%. The productions of IL-4 and IL-13 were seriously diminished by ESO that could also upregulate Treg cells by 22.2–97.1%. ESO has reduced the allergy response in mice by decreasing basophil degranulation, upregulating Treg cells via Forkhead box protein 3 (Foxp3), and releasing IL-10.

31.3.2 Anti-inflammatory Activity

Inflammation is a natural protective response of innate immune system to tissue injury or damaging external stimuli such as pathogens, allergens, infections, irritants, and ultraviolet light irradiation (Du et al. 2018). Inflammation involves a series of physiological responses against exogenous pathogens. Once infection is detected by the immune system, inflammatory signaling cascades recruit innate immune cells to the affected tissue sites. Immune cells, including macrophages, natural killer cells, and dendritic cells, use inflammatory signaling cascades to eliminate foreign substances such as viruses and bacteria (Chen and Nunez 2010). The immune system cells eliminate the pathogens by phagocytosis and production of cytokines and chemokines which play the vital roles in maintaining immune homeostasis. However, recent studies suggest that excess or chronic inflammation is associated with diseases such as inflammatory bowel disease, diabetes, cardiovascular disease, and neurodegenerative disease (Glass et al. 2010; Pearson et al. 2003); thus the regulation of excess and chronic inflammation is important for health. It is notable that chronic low-grade systemic inflammation can be induced by dietary factors like high-fat and high-sugar diets. Systemic inflammation subsequently affects local inflammatory responses and tissue-specific insulin resistance, which contributes to the progression of obesity, diabetes, and cancer. The incidence and progression of inflammation-related diseases can be prevented or delayed by lifestyle modifications, such as dietary intervention (Kang et al. 2016). In particular, the change of living habit and diet is an important factor for mitigating excess inflammation (Wu and Schauss 2012). Functional oligosaccharides can provide an effective way to prevent inflammation.

Sucrose-derived oligosaccharide is capable of regulating blood glucose and has great potential as an anti-inflammatory component in foods (Kang et al. 2016). The konjac oligosaccharide has markedly attenuated the inflammatory response to 2,4,6-trinitrobenzene sulfonic acid-induced experimental acute colitis (Liu et al. 2016). Comparing with its precursor, lactulose, oligosaccharides derived from lactulose have been characterized with slower fermentation by microbiota in the colon and showed beneficial effects in trinitrobenzene sulfonic acid-induced colitis rat. This kind of oligosaccharides would be able to reach the distal colon without great alterations. These properties could be of great interest in the treatment of

inflammatory bowel disease, because distal areas of the large intestine are typically affected in ulcerative colitis and involved very frequently in patients with Crohn's disease (Algieri et al. 2014). Oral administration of α-galacto-oligosaccharide (α-GOSg) and raffinose family oligosaccharides (RFOs) has significant protective effects against dextran sulfate sodium (DSS)-induced colitis. The beneficial effects of α-GOSg and RFOs were associated with the suppression of cyclooxygenase (cox-2) expression and possibly the other nuclear factor-kappa B (NF-κB)-related pro-inflammatory genes (Dai et al. 2018). Moreover, agaro-oligosaccharides (AGO) were found to be able to treat colitis through inducing the expression of heme oxygenase-1 in intestinal colonic mucosa. Also, increased colonic damage and myeloperoxidase activity after TNBS treatment was inhibited by AGO that can suppress the expression of tumor necrosis factor-α (TNF-α) induced by TNBS (Higashimura et al. 2013). D-galactose (DG) treatment mimicked the lung pro-inflammatory status as shown in the NA mice. FOS attenuated the DG-induced lung pro-inflammatory status and downregulated JNK/Jun pathway in the lung, which could be contributed to the probiotic and metabolic effects and metabolic products of FOS in the large intestine (Yeh et al. 2014).

The anti-inflammatory activities of HMOs and COS were reported. COS can inhibit inflammation and dyslipidemia in epididymal adipose tissue of diabetic mice (Zheng et al. 2018d). Synbiotics *Bifidobacterium infantis* and milk oligosaccharides (MO) are effective in reversing cancer-prone nonalcoholic steatohepatitis in western diet (WD)-fed FXR knockout mouse model (Jena et al. 2018). *B. infantis* and MO inhibited hepatic inflammation and reduced hepatic fat and liver injury, as well as normalize dysregulated bile acid synthesis and are useful to prevent NASH in WD-fed farnesoid x receptor knockout mice (Jena et al. 2018). Supplementation of infant formula with HMOs may be an important step toward improving the bioactivity of commercially available infant formula and promoting gut maturation and protection when mother's own milk is not available or insufficient (Rasmussen et al. 2017). The intestinal anti-inflammatory effect of goat milk oligosaccharides (OS) was assessed in trinitrobenzene sulfonic acid-induced colitis rat. Treatments fed with OS decreased in the anorexia and body weight loss, increased the trefoil factor 3 levels, reduced the bowel wall thickening and longitudinal extension of necrotic lesions, and downregulated the colonic expressions of interleukin 1β, inducible nitric oxide synthase, cyclooxygenase 2, and mucin 3. Thus, goat milk oligosaccharides have anti-inflammatory effects in rats with experimental colitis and may be useful in the management of inflammatory bowel disease (Daddaoua et al. 2006). HMOs also uniquely suit to provide optimal nutrition and immune protection to infants. Mice fed on 2′-fucosyllactose (2'-FL), one of the short-chain oligosaccharides associated with HMOs, have significantly increased vaccine-specific delayed-type hypersensitivity (DTH) responses accompanied by increased serum levels of vaccine-specific immunoglobulin (Ig) G1 and IgG2a in a dose-dependent manner. And increased activation marker (CD27) expression on splenic B lymphocytes was detected in 2'-FL-treated mice. Further, proliferation of vaccine-specific $CD4^+$ and $CD8^+$ T-cells, as well as interferon-γ production after ex vivo

restimulation, was significantly increased in spleen cells of mice receiving 2'-FL, which were in line with changes detected within dendritic cell populations (Xiao et al. 2018). COS also had some preventative effects such as alleviating atherosclerosis and enhancing the immune system. Treatment with COS can decrease the accumulation of lipid and significantly restore atherosclerosis in Apo E−/− mice and make a contribution to atherosclerosis prevention or treatment for elders in functional food (Zheng et al. 2018a). The murine model of the genital tract infected by *Chlamydia trachomatis* (Ct) was built to demonstrate the immune protective effects of chitosan oligosaccharides (COS) on mouse. Ten-day COS treatment increased the serum IgG antibody and IL-11 levels and spleen and thymus indexes, as well as reduced the positive infection rate and inclusion body formation with Ct, suggesting that COS can induce immune protection on the Ct-infected mouse genital tract and might be regarded as an alternative drug for the treatment of genital tract infection (Qian and Chen 2018). Alginate oligosaccharide (AO) administrated to the mouse model of ovalbumin-induced asthma showed that AO effectively decreased inflammatory cell numbers, eosinophil count, and IgE levels in BALF, and it dose-dependently inhibited asthmatic histopathological changes in the lung. AO can be a promising asthma treatment for its modulation of T-helper type 1 and type 2 cytokines (Bang et al. 2015). Zhang et al. have reported that COS treatment for 11 weeks upregulated the ratio of osteoprotegerin (OPG)/receptor activator of NF-κB ligand (RANKL) and downregulated the RANKL/RANK, thus improving the cartilage damage (both extent and grade) in OA male SD rats. COS may be used as a unique biological agent to prevent and treat osteoarthritis, and this effect is associated with modulation of the expression of osteoprotegerin and receptor activator of NF-κB ligand (Zhang et al. 2017). COS exposure led to a dose-dependent attenuation of LPS-induced secretion of TNF-α and IL-6 in the incubation medium. In addition, the corresponding reductions in TNF-alpha and IL-6 at the mRNA level indicated that exposure to these cytokines reduced the expression of these cytokines (Yoon et al. 2007). COS mitigates the glycerol-induced (acute renal failure) inflammatory response, improves renal function, and has the antioxidant effects in the kidney (Yoon et al. 2008; Fernandes et al. 2010). The anti-inflammatory effects of COS directly depend on their physical and chemical properties. The antioxidant and anti-inflammatory effects of enzyme synthesis depend on their degree of polymerization, while lower polymerization shows greater biological activity (Liang et al. 2016). Because it was observed to inhibit the expression of inflammatory genes caused by lipopolysaccharides (LPS), this was associated with reduced NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). Therefore, COS can weaken the vascular endothelial inflammatory response induced by LPS (Li et al. 2014). Low-molecular-weight COS shows protective effect via inhibiting lung inflammation in ovalbumin-induced allergic asthma mice. The oral administration of LM-COS can effectively alleviate the allergic inflammation in vivo and be a good source material for development of a potent therapeutic agent against mast cell-mediated allergic inflammatory responses and airway inflammation in allergic inflammatory diseases, including asthma (Chung et al.

2012). COS supplement not only attenuate organ dysfunction but also improve survival rate in LPS injection-induced sepsis mice (Qiao et al. 2011).

AC oligosaccharides (ACOS) show better inhibitory effects on LPS-induced mRNA expression of pro-inflammatory cytokines including IL-6, IL-8, IL-1, TNF, and MCP-1 in macrophage cells and suppressed the inflammation in lung tissues of C57BL/6 mice (Zheng et al. 2018c). The suppressive effect of ACOS on inflammation may be an effective approach to prevent inflammation-related diseases. The anti-inflammatory effects of a mixture of glucosamine and chitooligosaccharides (GC), produced by bi-enzyme single-step hydrolysis, are represented by the relievation of knee joint swelling and partial remission of typical OA symptoms in osteoarthritis (OA) mice. This study showed that GC had strong anti-inflammatory effects and potential as a therapeutic agent against OA and other inflammatory diseases (Li et al. 2018a).

31.3.3 Antidiabetic Activity

From International Diabetes Federation, type 2 diabetes used to be called non-insulin-dependent diabetes or adult-onset diabetes and accounts for at least 90% of all cases of diabetes. It is characterized by insulin resistance and relative insulin deficiency, either or both of which may be present at the time diabetes is diagnosed. The diagnosis of type 2 diabetes can occur at any age. Type 2 diabetes may remain undetected for many years, and the diagnosis is often made when a complication appears or a routine blood or urine glucose test is done. It is often, but not always, associated with overweight or obesity, which can cause insulin resistance and lead to high blood glucose levels. People with type 2 diabetes can initially manage their condition through exercise and diet. Contributing to the edible safety and pharmacological activity, prebiotics play a critical role in preventing diabetes mellitus and obesity. Prebiotics are usually nondigestible carbohydrates and can selectively promote the growth of beneficial bacteria. Some studies show that type 2 diabetes and obesity are related to the dietary fiber intake. The dietary fiber can control the release of glucose into the blood with time and is conducive to the management of diabetes mellitus and obesity (Bennett et al. 2006). Functional oligosaccharides are considered as the important source of dietary fibers (de Alcantara et al. 2006), which can modulate satiety, glucose or lipid metabolism, and hypertension. The functional oligosaccharides have been discovered to reduce postprandial blood glucose and insulin responses and improve the overall blood glucose and lipid concentrations. The arabinoxylan oligosaccharides can prevent type 2 diabetes by reducing postprandial blood glucose levels as well as increasing insulin sensitivity and postprandial ghrelin secretion (Xu et al. 2009). FOS was reported to promote the growth of beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Bacteroides*. Besides, it's benefit to diabetic individuals (Zheng et al. 2018b). Research on antidiabetic activity of oligosaccharides has received wide attention. A partially purified oligosaccharide from Rehmanniae Radix was shown for the first time to be an active principle of prepared Rehmanniae Radix that improved glucose intolerance in type 2

diabetic mice (Chiu et al. 2018). COS improve the disturbance in glucose metabolism and reverse the dysbiosis of gut microbiota in diabetic mice (Zheng et al. 2018b). Mannan-oligosaccharide (MOS) supplementation on C57BL/6 J mice fed a high-fat diet (HFD) results in attenuated high-fat diet-induced metabolic syndrome, including slower body weight gain, lowered serum lipids, and reduced insulin resistance (Wang et al. 2018b). MOS also modulate the overall structure of the gut microbiome, which is highly correlated with metabolic syndrome parameters (Wang et al. 2018a). *Ophiopogon japonicus* oligosaccharide (OJOS) treated to *db/db* diabetic mice for 8 weeks could effectively lower the body weights, blood glucose levels, and the AUCs during oral glucose tolerance test and inhibit the changes of 24 h urinary albumin excretion and 24 h urine protein amounts, glycosylated serum protein, total cholesterol, triglyceride, HDL-C and low-density lipoprotein cholesterol, and urea nitrogen and creatinine levels in serum, suggesting that OJOS shows significant hypoglycemic and protective effects in *db/db* mice (Wu et al. 2017). To study the anti-obesity and antidiabetic effects of neoagarooligosaccharides (NAOs), mice were fed with HFD + NAOs (0.5%, w/w) (HFD-0.5 group) for 64 days, which showed significant reduction of 36% for body weight gain and 37% for food efficiency ratios without abnormal clinical signs compared to the HFD group. Fat accumulation in the HFD-0.5 group was recovered nearly to the levels found in the normal diet group. NAOs also significantly decrease the size (area) of adipocytes and tissue weight gain in the perirenal and epididymal adipose tissues. After NAOs were absorbed by mice fed with HFD, the increased concentrations of total cholesterol, triglyceride, and free fatty acid in serum were also effectively ameliorated to the levels found in serum of the mice fed with normal diet. Also, insulin resistance and glucose intolerance in mice with HFD were distinctly improved, and adiponectin concentration in the blood was notably enhanced after NAO administration. Intake of NAOs can effectively suppress obesity by inducing production of adiponectin in the HFD-induced obese mice (Hong et al. 2017).

Low-molecular-weight chitosan oligosaccharide (GO2KA1) remarkably inhibited sucrase in all three parts of the intestine, while glucoamylase activity was decreased only in the middle and lower part. Sucrase, as a digestive enzyme secreted in the small intestine, catalyzes the hydrolysis of sucrose to its subunits fructose and glucose. The sucrase-isomaltase (SI) complex expression on mRNA level was evaluated; GO2KA1 had minimal inhibitory effect on the upper part and more pronounced inhibitory effect on the middle part, while the highest inhibition was observed on the lower part. Long-term GO2KA1 supplementation in mice shows significant blood glucose and HbA1c reduction, confirming the previous in vitro observations that GO2KA1 has inhibitory effect on carbohydrate hydrolysis enzymes, namely, sucrase or maltase (Kim et al. 2014).

31.3.4 Anticancer Activity

Cancer is one of worst nightmares for human, increasing with changing lifestyle, nutrition, etc. According to the report, cancer belongs to the large group of complex,

devastating diseases that entail uncontrolled cell growth with the potential to invade or affect any part of the body. Death due to cancer worldwide is projected to rise with an estimated 12 million deaths in 2030. Around 25 million persons are living with cancer around the world (Foster and Fenlon 2011). Therefore, cancer is a painful existence for the public. The formation of cancer cells in human body can be directly induced by free radicals. Multiple natural anticancer drugs like chemopreventive agents have gained positive effects on cancer treatment (Gurib-Fakim 2006). Oligosaccharides as a nature resource can be used indirectly to reduce cancer formation in human body. Recently, numerous studies reported that oligosaccharides have antiproliferative activity in cancer cell lines in vitro, as well as inhibitory activity against tumors growing in mice. Ginseng oligosaccharides can reliably inhibit the growth of mouse transplantable H22 cells and improve the weight of immune organs of tumor-bearing mice (Jiao et al. 2014). The antitumor and immunoregulatory effects of water-soluble ginseng oligosaccharides (WGOS) were further evaluated in hepatoma-22 (H22)-bearing mice, which demonstrated that treatment with WGOS inhibited tumor growth in vivo and significantly increased relative spleen and thymus weight, serum tumor necrosis factor-α level, spleen lymphocyte proliferation, natural killer cell activity, phagocytic function, and nitric oxide production secreted by macrophage in H22-bearing mice (Jiao et al. 2014). Chen et al. (2011) found that the Lewis X oligosaccharides-heparanase complex could be regarded as an ideal vaccine and represented a novel way for the therapeutical strategy of tumor. Agaro-oligosaccharide (AGO) feeding resulted in tumor appearance delay and tumor number decrease in the two-stage mouse skin carcinogenesis model. And prostaglandin E2 (PGE2) production was suppressed by AGO intake in 12-*O*-tetradecanoylphorbol-13-acetate-induced ear edema model. AGOs are expected to prevent tumor promotion by inhibiting PGE2 elevation in chronic inflammation site (Enoki et al. 2012). Carrageenan, extracted from marine red algae, has been largely applied as nutrient supplements and biochemical reagents and in medicine. The sulfonated and non-sulfonated carrageenan oligosaccharides were assayed for the antitumor activity in sarcoma 180 tumor in mouse. After 15 days of transplantation, the weight of tumor in mice increased to 1.248 g. When the mice were provided with anticancer drug cyclophosphane by intramuscular injection, the weight of tumor decreased to 0.184 g, and the rate of tumor inhibition came to 85.3%. For the non-sulfonated (sulfur content 1.0 per disaccharide unit) and light-sulfonated (sulfur content 2.1) oligosaccharides, the weights of tumor decreased to 0.442 g and 0.272 g, and the rates of tumor inhibition reached 64.6% and 78.2%, respectively, in the dose of 100 mg kg^{-1} (Mou et al. 2003; Yeh et al. 2014). Water-soluble oligosaccharides were extracted from the fruiting bodies with water and then used to examine the anticancer activitis in mice. Gray oligosaccharide (LDGO-A) has potential to be the compounds of natural antitumor by which the tumor inhibitory rate can reach to 40.02% and the tissues arrange more regular and firmer in histology of immune organs (Ding et al. 2015). Mice treated with chitosan (5 mg/kg/day) in combination with *Agaricus blazei* Murill (246 mg/kg/day) for 6 weeks have reduced levels of glutamic oxaloacetic transaminase (GOT) and vascular endothelial growth factor (VEGF) (Yeh et al.

2015). And, N-acetyl-d-glucosamine oligomer (NACOS) and glucosamine oligomer (GO) have significant effect on suppressing tumor growth and lead to tumor tissue apoptosis apparent in a tumor (colon 26)-bearing mouse model, which can be candidates as antitumor functional foods (Masuda et al. 2014).

Recently, many researchers have reported that the hydrolyzed products of chitosan N-acetyl-d-glucosamine oligomers (chitin oligosaccharide; NACOS) and D-glucosamine oligomers (chitosan oligosaccharides; COS) possess numerous biology activities (Azuma et al. 2015) and the chemopreventive effect of COS in colon cancer cells (Nam et al. 2007). The treatment effects were evaluated measuring the activities of enzymes quinone reductase (QR), ornithine decarboxylase (ODC), glutathione S-transferase (GST), and glutathione (GSH) in human colorectal adenocarcinoma cell line ht-29 (cox-2). COS had a chemical effect on colon cancer by increasing QR and GST activities and GSH levels, as well as by inhibiting ODC activity and cox-2 expression (Azuma et al. 2015). COS was reported to slow down the growth of tumors in mice (Zhai et al. 2018). COS may also be beneficial in suppressing colorectal cancer development, and a progressive disease can be detected early by colonoscopy (Mattaveewong et al. 2016). COS also have bioadhesive characteristic (Tokumitsu et al. 2000). Good adhesion was found in epithelial tissue and mucous coats on the tissue surface. The positive charges of the COS can alter the ion environment of the cell membrane, which is important to maintain cell integrity and many of the functions necessary for cell growth (Zou et al. 2016). A negative charge on the surface of a tumor cell can independently trigger its signal transmission. As a result, malignant biological behaviors such as invasion and metastasis occur. COS are the only natural polysaccharides that have a cationic charge. Therefore, the electrostatic effect is proposed to help absorb the COS on the cell membrane of tumor cells and change the charge properties, thus changing the permeability of tumor cells. Conversely, the mutual repulsion between the COS and normal cells has the same positive charge resulting in selective targets for tumor cells other than normal cells (Huang et al. 2006). Matrix metalloproteinase-9 (MMP-9) is vital components in cancer invasion and metastasis. The upregulated expression of MMP-9 leads to the release of vascular endothelial growth factor (VEGF) and formation of angiogenesis. COS can suppress tumor angiogenesis by inhibiting the expression of MMP-9, which consequently suppresses the expression of VEGF (Xu et al. 2012). It was suggested that tumor cells were not killed directly by COS but indirectly related to the immune stimulation effect of COS. This increases the production of lymphocytes, induces lymphocytic cytokines, and promotes the proliferation of lysocytic lymphocytes, thus producing antitumor effect (Tokoro et al. 1988).

31.3.5 Antiaging Activity

The function of COS in skin has attracted increasing attention in recent years for its skin-permeable and moisturizing abilities. COS can markedly suppress the UVB-induced increase in mRNA and protein expression of matrix

metalloproteinases-1 (MMP-1) and matrix metalloproteinases-3 (MMP-3) in skin fibroblasts. Hence, the deeper mechanism of antiaging is explored by researchers. The experiment of anti-photoaging activity gradually came into research's view. Topical application of COS contributed to the prevention of ultraviolet-induced skin dryness, epidermal hyperplasia, and wrinkle formation in mouse skin manifested by increasing activities of antioxidative enzymes and suppressing the production of pro-inflammatory cytokines, which presumably worked in concert to inhibit the excessive degradation of collagen (Kong et al. 2018a). COS also has a protective effect against D-gal-induced damages of subacute aging in mice mainly by promoting the serum IgG and IgM levels, preventing the atrophy of mice thymus and spleen, as well as increasing the activities of pivotal antioxidant enzymes in mice liver and kidney, revealing that the mechanism might be closely associated with its beneficial modulation of oxidative and immune system (Kong et al. 2018b).

31.3.6 Anti-obesity Activity

Obesity is one of the most important public health problems, with more than 2.6 million people dying each year from obesity and its related complications. More than 1.7 billion adults worldwide have excess weight among which 312 million are obese (Ejtahed et al. 2018). With the social and economic development, everyone's pace of life has changed a lot compared to the past, especially in the diet. Unhealthy eating patterns can cause a range of diseases. Recent research has shown that the gut microbiome is a new factor in obesity and related metabolic risk in humans (Blandino et al. 2016). Excess calories, mainly stored in fat pads, cause the systemic deregulations. Continuous supply of these calories may further get stored to the non-adipose tissues in the form of fat and called as ectopic fats that further promote insulin resistance and nonalcoholic fatty liver. Adipose tissue hypertrophy/hyperplasia recruits macrophages and releases inflammatory cytokines leading to low-grade systemic inflammation (Singh et al. 2017, 2018). Fucosylated chondroitin sulfate oligosaccharides from *Isostichopus badionotus* regulate lipid disorder in C57BL/6 mice fed a high-fat diet (Li et al. 2018b). COS injection lead to reduce the dietary intake, body weight, and glucose and lipid levels, which may act as a potent downregulator for expression levels of obesity-related genes in *ob/ob* mice. *Rehmannia glutinosa* oligosaccharides exhibited strong protective effects against high l-carnitine-induced liver injury in mice (Li et al. 2016). COS also reduce plasma total cholesterol (TC), aortic lipid deposition, and body weight representing a clear function in regulating blood lipids (Muanprasat and Chatsudthipong 2017). Cyclodextrins (CDs) has same function as well. CDs are cyclic oligosaccharides that have been generated from starch by enzymatic cleavage of the amylose helix, of which the three most studied species consist of six, seven, or eight glucopyranose units called α-CDs, β-CDs, and γ-CDs, respectively (Davis and Brewster 2004). In particular, the β-CDs are able to interact with cell membranes and are known to extract cholesterol and other lipids from the membranes (Irie et al. 1992; Ohtani et al. 1989), and CDs can extract cholesterol from the plasma membrane, and then, cholesterol from the

intracellular compartments moves to the plasma membrane to replace the lost cholesterol. Also, CDs can enter the cell via endocytosis and directly bind and export cholesterol from the endolysosomal compartments (late endosomes and lysosomes), and CDs, either at the plasma membrane or after endocytosis, can induce cellular signaling pathways that promote intracellular cholesterol (Szente et al. 2018). Bovine milk oligosaccharides and *Bifidobacterium longum* subsp. *infantis* jointly restore diet-induced obesity intestinal microbiota and barrier function defects in mice (Boudry et al. 2017). And isomalto-oligosaccharides, green tea extract, and their combination exerted beneficial effect against high-fat diet-induced negative metabolic (gut and adipose tissue) and nonalcoholic steatohepatitis phenotype primarily by preventing dysbacteriosis and resultant endotoxemia (Singh et al. 2017, 2018). Chitosan oligosaccharide capsules (COSCs) possess anti-obesity effect via activating the JAK2-STAT3 signaling pathway to alleviate leptin resistance and suppressing adipogenesis to reduce lipid accumulation on rats suffering from obesity induced by a high-fat diet (Pan et al. 2018b). Fatty acid synthesis and blood triglyceride content were effectively reduced after galacto-oligosaccharides (GOS) feeding which may improve lipid metabolism in mice (Cheng et al. 2018). Additionally, NACOS can improve glucose metabolic disorder and suppress mRNA expression of the protein regulators related to lipogenesis, gluconeogenesis, adipocyte differentiation, and inflammation in adipose tissues, as well as inhibit the destruction of the gut barrier in HFD-treated mice (Zheng et al. 2018b). One study investigates the anti-obese effects of glucosamine (GLC) and COS on high-fat diet-induced obese rats. The result demonstrates that GLC and COS improve dyslipidemia and prevent body weight gains by inhibiting the adipocyte differentiation in HFD-fed rats (Huang et al. 2015).

31.3.7 Promoting Digestion Function and Gut Health

The intestinal microflora plays an important role in the metabolism of endogenous and exogenous substances in the diet and is thought to participate in beneficial and deleterious effects on human health. Fermentability and viscosity are the two main characteristics of dietary fibers affecting energy intake weight gain, fat accumulation, and the lipid profile (Hadri et al. 2015; Kristensen and Jensen 2011). At present, fermentable fibers such as FOS have a potent short-term inhibitory effect on food intake and promote a decrease of body weight. These effects of fermentable fibers are thought to be related to their modulation of gut microbiota and to the production of short-chain fatty acids (SCFAs) which act as indirect regulators. Functional oligosaccharides can promote the increase of short-chain fatty acids. According to the studies, constipation can be affected by the composition of the microbiome and its metabolic activity (Liebregts et al. 2007). Fructo- and galacto-oligosaccharides are known to stimulate the growth of *Bifidobacterium* and *Lactobacillus* and also inhibit potentially pathogenic bacteria, enterobacteria, *Clostridium difficile*, and *Salmonella* (Xu et al. 2009). Besides, short-chain fatty acids produced by *Bifidobacterium* can stimulate intestinal peristalsis and improve fecal humidity

through osmotic pressure, thus playing a role in the treatment of intestinal diseases (Fernández-Bañares 2006). It can improve the absorption of water and electrolytes in the small intestine, thereby leading to a reduction in the incidence of diarrhea and its duration. Short-chain fatty acids are an important source of energy for enterocytes and are key signaling molecules for the maintenance of gut health. In the case of newborns, the gastrointestinal tract is a vital part of their immune system. The intestinal epithelium constitutes a functional barrier that prevents pathogenic invasion of the host through the gut microbiome and provides the active components of the lymphocyte lineage that modulate the immune response (Lewis et al. 2017). The immature intestinal tract of neonates has excessive expression of inflammatory genes and insufficient expression of genes regulated by congenital signal feedback. This can lead to an exaggerated immune response leading to excessive inflammation. Breast feeding can substantially alter the neonatal intestinal immunity (Newburg and He 2015). Studies show that the human milk oligosaccharides can indirectly increase the production of short-chain fatty acids, and these augmented levels are mediated by *Bifidobacterium* species. SCFAs can interact with the receptors GPR41 and GPR43, increasing the intestinal secretion of polypeptide YY and glucagon-like peptide 1 (GLP-1), respectively (Canfora et al. 2015).

FOS supplementation results in the enlargement of the cecum and increased cecal acidification. FOS also effectively boosts the apparent absorption rate of calcium, magnesium, and iron, as well as restores the phytic acid-impaired magnesium and iron apparent absorption rates, additionally significantly increasing hepatic zinc levels and femoral magnesium levels (Wang et al. 2010). Dietary supplementation of 2 g/kg COS can increase the growth performance of weaning pigs by increasing dry matter and nitrogen digestibility and modulating the frequency of diarrhea (Zhou et al. 2012). It has been reported that the inhibitory effect of dietary fiber on food intake can be lost over time and that FOS gum limits fat storage (Hadri et al. 2015). NAOS can be effectively resistant to enzymes of the upper gastrointestinal tract, which remained intact after 24 h incubation with different amylolytic enzymes. NAOS remarkably stimulated the growth of *Bifidobacterium* and *Lactobacillus* in Man-Rogosa-Sharp (MRS) medium, anaerobically, and significantly increased the numbers of *Lactobacillus* and *Bifidobacterium* ($p < 0.05$) in fresh feces or cecal content while reducing putrefactive microorganisms in vivo. Mice fed with 2.5% (w/v) NAOS for 7 days had larger increases in colonic beneficial bacteria population than those fed with even 5% (w/v) FOS for 14 days. NAOS may be a great prebiotic for host (Hu et al. 2006). Co-supplementation of cranberry extract (CRX) and isomalto-oligosaccharides (IMOs) significantly improved cecal SCFA contents, gut beneficial bacterial quantity, gut histology, and related changes (colon mucin production, gut permeability) as compared to individual agents in HFD-fed mice. It also prevented HFD-induced systemic and tissue inflammation, glucose intolerance, and systemic obesity-associated metabolic changes in the adipose tissue and liver. The combination of CRX and IMOs appeared more effective in the prevention of HFD-induced gut derangements (Singh et al. 2017, 2018). The low-molecular-weight chitosan may have a therapeutic effect on osteolytic diseases for its inhibition effect on skull bone damage in LPS-induced mice (Guo et al. 2018). The mixture of

oligosaccharides and other substance has tremendous potential. A novel prebiotic blend (PB) composed of FOS, GOS, inulin, and anthocyanins can effectively prevent the development of irritable bowel syndrome (IBS) by attenuating the inflammatory response both in Caco-2 cells and the post-infectious IBS model. And pretreatment of PB in mice effectively reduces the severity of the IBS symptoms and modulates the gut microbiota in the PI-IBS model. This provides biological plausibility to the usefulness of this prebiotic product as a preventive measure for gastrointestinal dysbiosis and dysfunction (Chen et al. 2017). Another study shows the combination of IMOs with cinnamaldehyde presents untoward effects on the resident gut microbiota and associated metabolic outcomes in HFD-fed mice (Singh et al. 2017, 2018).

Human milk oligosaccharides (HMOs) consist of free oligosaccharides and have high structural diversity and represent the third largest group of bioactive molecules in human milk (Kulinich and Liu 2016). The vast majority of HMOs contain a lactose, polylactosamine, or lacto-*N*-biose core. Galactose, glucose, fucose, *N*-acetylglucosamine, and the sialic acid derivative, *N*-acetyl-neuraminic acids, are the five monosaccharide building blocks that can constitute HMOs (Kobata 2010). All HMOs carry lactose (Galβ1-4Glc) at the reducing end, which can be elongated in a beta-1,3 or beta-1,6 linkage by two different disaccharides, either type 1 carbohydrate structures (containing Galβ1-3GlcNAc units) or type 2 structures (containing Galβ1-4GlcNAc units) (Zhao et al. 2017). Because humans lack the enzymes (sialidases or fucosidases) to break down HMOs, these compounds reach the colon intact where they are digested by bacteria within the intestinal microbiota (Plaza-Diaz et al. 2018). HMOs are considered as prebiotics to promote the growth of a favorable microbiota. HMOs can induce increased levels of *Bifidobacterium* in the colonic flora of breast-fed infants, accompanied by a great reduction in pathogenic potential bacteria by the bifidogenic activity of HMOs (Moon et al. 2016; Thomson et al. 2018). HMOs have a great influence on the composition of intestinal flora. Therefore, we can selectively use some *Bifidobacterium* to produce beneficial effects on the human body (Zhao et al. 2016).

31.3.8 Protective Liver Function

Alcohol abuse is one of most important health issues, as it causes 3.8% of all deaths (Rehm et al. 2009). The alcohol excessive consumption is also a major cause of liver disease as it could trigger serious liver injuries and result in liver fibrosis and cirrhosis. According to the reports of US National Institute on Alcohol Abuse and Alcoholism, alcohol-induced liver disease accounts for up to 48% of cirrhosis-related deaths in the United States and is the leading cause of death from liver injury-related deaths in other countries (Gao and Bataller 2011). In addition, although the acetaminophen (*N*-acetyl-*p*-aminophenol, APAP) as an antipyretic and analgesic drug is safe when taken at prescribed doses, an overdose can induce severe hepatotoxicity in both humans and experimental animals (Thomas 1993). APAP is primarily metabolized in the liver by phase II conjugating enzymes, mainly UDP-glucuronosyltransferase (UGT) and sulfatase, to generate the nontoxic

metabolites APAP-glucuronate and APAP-sulfate (Thomas 1993). *N*-acetyl-*p*-benzoquinone (NAPQI) as the cytochrome P450-mediated metabolic APAP metabolites can cause liver damage, whose toxicity binds to cell macromolecules such as proteins, lipids, and DNA (James et al. 2003). NAPQI also reacts with glutathione (GSH), causing cell GSH failure and producing reactive oxygen species (ROS) in the liver. In the case of APAP overdose, the induction of hepatic antioxidant enzyme activity, such as NADPH quinone oxidase 1 (NQO1) and heme-1 (ho-1), may be an adaptive response to counteract the imbalance of antioxidant homeostasis in the liver, thereby reducing ROS formation and limiting the progression of hepatotoxicity (Aleksunes et al. 2006). Natural products that decrease cytochrome P450 enzyme activity (Mutlib et al. 2006) and increase antioxidant enzyme activity or GSH levels may attenuate APAP-induced liver toxicity (Acharya and Lau-Cam 2010). In vitro and in vivo studies have shown that COS can alter drug-metabolizing enzyme (DME) and antioxidant system. COS has been shown to increase the activity of the second-stage detoxification enzymes such as NQO1 and glutathione S-transferase (GST) enzyme (Nam et al. 2007) and to induce intracellular GSH level (Mendis et al. 2007). At the same time, COS has been demonstrated to have hepatoprotective activity against chemically induced liver injury (Chen et al. 2005).

31.3.9 Calcium Absorption Promotion

Calcium is an essential nutrient in bone hydroxyapatite, and an adequate dietary intake of calcium during childhood and adolescence is important for mineralization of the skeleton and achievement of genetically programmed peak bone mass (Sanwalka et al. 2012). Low-calcium intake in adolescent girls is associated with an increased risk of bone fractures later in life (Abrams et al. 2004). Therefore, optimal calcium intake is very important during adolescence. Most of calcium absorption occurs in the small intestine. However, if the insoluble, unabsorbed calcium coming from the small intestine is maintained in an ionic form, about 5–10% could occur in the colon (Younes et al. 1996). Nondigestible FOS is not hydrolyzed enzymatically in the small intestine but is fermented by resident microbiota in the colon, producing SCFAs that lower intraluminal pH and thereby may increase mineral solubility (Cummings and Macfarlane 2002). In addition, the low pH modifies the colonic microbiota and increases proliferation and activity of beneficial flora, such as *Lactobacillus* species (Macfarlane et al. 2006). However, even a proper diet does not guarantee a proper calcium balance. In addition to quantity, its absorption is a key factor in determining the biological availability for bone development and maintenance. In some cases, poor calcium balance may be due to intestinal malabsorption due to infection, inflammation, or pathology. Some people in the calcium-deficient population have chronic bowel diseases, including celiac disease (Krupa-Kozak et al. 2017). Inulin, a polydisperse carbohydrate material consisting mainly of beta (2–1)fructosyl-fructose links, is an example of prebiotic naturally occurring in tubers, bulbs, and tuberous roots of several edible fruits and vegetables (Drabinska et al. 2016). Inulin and other inulin consumption

also lead to the production of SCFAs. On the other hand, they stimulate the growth and/or activity of selected symbiotic bacteria, including health-promoting *Bifidobacterium* and *Lactobacillus* (Rossi et al. 2005). Moreover, inulin and oligofructose are not hydrolyzed by the human digestive enzymes nor absorbed in the upper part of the intestinal tract (Ellegard et al. 1997). Following ingestion of a few grams per day of inulin or oligofructose, the composition of the fecal flora of human volunteers is significantly modified: *Bifidobacterium* becomes the dominant genus, and potentially harmful species such as clostridia are decreased (Roberfroid et al. 1998). In human trial, scientist has validated a stable isotope technique to determine the true intestinal calcium absorption allowing to distinguish the exogenous calcium and endogenous calcium, thus measuring true calcium absorption. These measurements were based on the amount of calcium isotopes in urine after 24 h of collection. There was no significant difference in mineral uptake among healthy young adults supplied with a 15-gram/day placebo, inulin, or oligofructose, respectively, in this study. If the large intestine is the major place in which fructans enhance calcium absorption, a 24-h period of urine collection may be too short however to detect the effect. This experiment at least confirmed that fructans have no adverse effects on calcium absorption (Franck 2006). In the basis of these results, researcher performed a similar study but this time with a group of male adolescents and collected urine sample about 36 h instead of 24 h. Adolescents were chosen because it is thought that the mineral absorption rate is highest during adolescence. Twelve volunteers consumed 15 g/day of either oligofructose or a placebo (sucrose) for 1 week. A significant increase (+26%; $p < 0.05$) in the fractional calcium absorption from 47.8% (placebo) to 60.1% (oligofructose) was observed upon ingestion of oligofructose. This evidence indicates that oligofructose may help to maximize the peak bone mass in adolescents (Franck 2006).

31.4 Application in Foods

The current trend shows the demand for the functional foods has increased vigorously. Among this category, the functional oligosaccharides are beneficial ingredients implied in productive process. What's more, the oligosaccharides derived from plant, animals, and microbial sources were widely used in daily food, beverages, bakery products, processed fruits/vegetables, confectionary, infant formulas, and meat industry. With the extensive use of functional oligosaccharides, the annual production of oligosaccharides has been increased annually. There were about 20 kinds of oligosaccharides that were put into quantity production. Japan and Europe were the leading manufacturing market to produce oligosaccharide. Both of them have produced the oligosaccharides on a mass-factory scale 35 years ago, especially the production of fructo-oligosaccharide (Panesar et al. 2018). Besides, Europe in 2015 had accounted for the biggest market in the production of oligosaccharide which was added to infant formula. According to the data showed in related literature, the market value of prebiotics reached $1.17 billion in 2015, which indicated that the oligosaccharide contained within prebiotic would bring high

profits. And with the increasing demand for prebiotic, the market profits would be substantial (McDougall 2011). In 2011, the demand for functional oligosaccharide around the world reached up to 1.35 million tons. In 2010, the demand mass of isomalto-oligosaccharide (10,000 tons) was the most in China, the demand quantity of fructo-oligosaccharide (80,000 tons) was second, while the gross product in China was only 12,0000 tons which couldn't satisfy the numerous requirement of the society.

Nowadays, along with the alteration of people's diet concept, the public focused not only on tastes of food but also on the function of food. Meanwhile, the fructo-oligosaccharide (FOS) could serve as the flavoring agent and nutrient supplements in food. The sweetness of fructo-oligosaccharide is 0.4–0.6 times sweeter than sucrose, so it is used as full-fat ice cream at 10% and 30% sucrose replacement levels with minor effect on the apparent viscosity and pseudoplasticity (Soukoulis et al. 2010). Yogurt is well populated in Mediterranean, Asia, and Central Europe market, but the sour taste of yogurt can be produced by *Streptococcus thermophiles* and other acid-forming bacteria in the process of production. The addition of FOS can mask the sour taste to enhance acceptability of products (Irvine and Hekmat 2011). What's more, it plays a vital role in fermentation time and viability of the probiotic strain during storage (Singla and Chakkaravarthi 2017). The laxative effect and sensory of biscuits added with FOS was enhanced. In the meat industry, FOS was used to improve technological parameters. Especially, addition of short-chain FOS not only has no effect on weight loss and water activity but also reduced the energy with 92 kcal/100 g (Bali et al. 2015). Huet et al. (2016) had found that partly fermented infant milk formulae containing short-chain GOS and long-chain FOS were well-tolerated and had no side effect on the growth of healthy infants. The fructo-oligosaccharides were also used in fruit juice and act as fortification to improve the quality of product (Renuka et al. 2009). What's more, the addition of FOS in frozen dough improved the baking quality even after frozen storage for up to 8 weeks. The added FOS alleviated deterioration of frozen dough and thus resulted in increases in proof volume and loaf volume of bread and decrease in hardness of bread crumb. Thus it can be used in bakery food to improve edible quality (Park et al. 2016). With the extensive use of FOS, the market value of FOS is a considerable figure. FOS was firstly researched and developed by Meiji Japan, which is one of the leading manufacturers. In 2007, the FOS sales volume in Japan was 3,000 tons and kept a 12.8% market share of functional oligosaccharides. And Japan sales accounted for 24.72% of the global market share. China had a late starting for the exploitation of FOS, but the productivity ranked top among the world with the productive mass for 15,000 tons.

Due to the nondigestible and low-cariogenic feature of galacto-oligosaccharide, it was applied in beverage, infant milk formula, functional food, and baked goods (Czermak et al. 2004). The addition of GOS to yogurt can produce smoother texture and not break down this prebiotic in the gut. GOS is also applied in beverage because it can clear solutions and sustain high processing temperatures during machining process. Its low glycemic index makes it appropriate for patients suffering diabetes or cardiovascular disease (Sangwan et al. 2011). Besides, GOS can selectively

stimulate the growth of probiotic in the gut which can be exploited by pharmaceutical (Torres et al. 2010). Japan was the first country to put the galacto-oligosaccharide (GOS) into commercial process with the sales figure $200 million, and the sales in world reached up to $1,000 million in 2004. Further, the yearly output of GOS in the worldwide market was 94.1 kilotons and is expected to increase to 175.7 kilotons by 2020. Moreover, the demand of GOS in China market also increased annually (Panesar et al. 2018).

Inulin is a common prebiotic, which was firstly extracted from the tuber of inula in 1804, and was added in bakery products to encapsulate starch granules, thus lowering their digestion rate. In addition, inulin can improve the color of white bread through Maillard reaction (Poinot et al. 2010). With the increasing demand for calorie-controlled and healthier food, various food industries were forced to derive few alternative safe sweeteners for consumption. The use of inulin as a fat replacer in ice cream with levels of 9% results in enhanced consistency and stickiness (Akbari et al. 2016). To exploit a palatable and nutritive meat product, inulin is supplemented in minced meats as fat replacer with reduced fat content, and the color, texture, and flavor are similar to the meats without inulin (Rodriguez Furlan et al. 2014). Calories from the fermented sausage were significantly reduced on 10% inulin addition. The short-chain inulin which contains various kinds of saccharides was widely used for the production of inulin oligosaccharides (IOS). Inulin and resistant starch could be used to take the place of 7.2% of fat in imitation Mozzarella cheese (Bi et al. 2015). At present, there is only three commercial manufacturing companies (Orafti, Warcoing, and Sensus) producing inulin. The Beneo Orafti Co., which is the member of Sudzucker group, lies in Belgium and is also the biggest inulin manufacturing enterprise around the world. The annual output of inulin is up to 400,000 tons with the annual yield for approximately $0.7 billion. Orafti also uses inulin as raw material to produce IOS and FOS. Warcoing Co. is also located in Belgium, which was the first company to produce inulin. The output of inulin comes up to 100,000 tons with a market segment of 50%. Sensus Co. is the subsidiary company of Cosun Group, which is seated in the Netherlands. The annual sales revenue is up to $1.26 billion. Above all, Europe occupies the majority of inulin market. Besides, the Innova Market Insights data show that inulin has the highest market share of 22% in dietary fiber-containing food and beverage in 2017. And the compound annual growth rate (CAGR) of inulin industry is estimated to reach 9.1% from 2015 to 2022. The global market size is expected to total $2.37 billion. The application and effect of FOS, GOS, and inulin is present in Fig. 1.

A mixture of FOS, GOS, and inulin is also a common ingredient applied in food (Fig. 2). For example, the mixture of FOS and inulin has been incorporated in edible coatings of apples to enhance the nutritional benefits and shelf life of freshly cut apple by reducing the water loss and selectively controlling gas permeability (Rößle et al. 2010). Besides, the use of FOS/inulin (50:50) can enhance the acceptability of *Lactobacillus acidophilus* and *Bifidobacterium animalis* contained in petit-suisse cheese and increase the content of linoleic acid in cheese (Rodrigues et al. 2012; Cardarelli et al. 2008). The addition of inulin and FOS improves the appearance and mouthfeel of orange cake with brown color and chewiness (Volpini-Rapina et al. 2012).

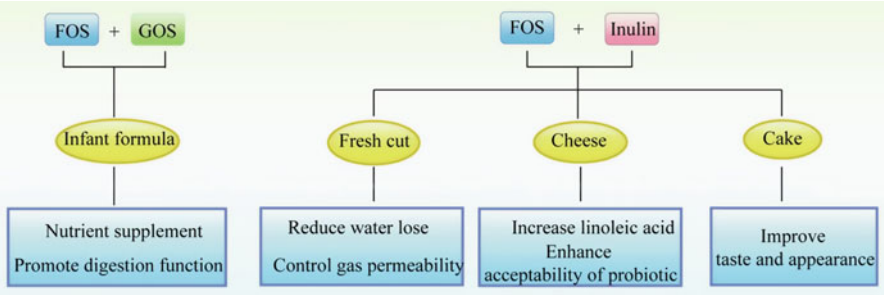

Fig. 2 The application of the mixture containing GOS, FOS, and inulin

Chitosan oligosaccharide (COS) from the acidolysis of chitin has plenty of potential for an extensive range of application. Due to its antimicrobial activity against pathogenic and nontoxicity for organism, it can be formed into films for the use of packaging material to improve the quality of food (Dutta et al. 2012). What's more, COS can play a vital role in modulating the status of intestine, so it is indispensable for the production of yogurt (Qin et al. 2014). China was the coastal country which has abundant marine sources for the production of COS. The annual output of COS is maintained in a high level with the yield for 2,000–3,000 tons in 2014. At present, the selling price of COS was about $118–145 which means the oligosaccharide implied the huge profits. Soybean oligosaccharide (SBOS) was the general term for several soluble oligosaccharides, such as sucrose, stachyose, and raffinose (Guillon and Champ 2002). In 2011, because of the extensive cultivation of soybean, the yield of SBOS in China was 10,000 tons which was only next to America. And SBOSs were used in beverages, yogurts, infant formula, and bakery goods.

As the golden standard, breast milk is tailored by mothers to provide their newborn babies with essential nutrients (Akkerman et al. 2019). But not every mother has adequate breast milk for their baby, and there were about 60% mothers who need to work after delivery. HMOs are the third most abundant components in breast milk, which are precious nutriments that no other normal milk can offer. Some synthesized oligosaccharides were added to infant formulas to mimic the breast milk, such as lacto-*N*-neotetraose (LNnT) and 2'-*O*-fucosyllactose (2'-FL) which were applied to infant and toddler formula in Europe and the United States. However, since there are limited source of HMOs and insufficient technology for commercial generation, the extensive use of HMOs in market was blocked (Grabarics et al. 2017). In 1930, the HMOs were firstly extracted from human milk, and with the following years, numerous experiments were carried out to identify the structure and function of HMOs (Plaza-Diaz et al. 2018), and the research on HMOs becomes a hot topic and is associated with the production method since 2010. Health benefits associated with the consumption of human milk oligosaccharides include maintaining gut microbiota and inhibition of pathogen in infants. These are factors likely to drive the growth of the global human milk oligosaccharide market.

Human milk oligosaccharides are jumping into popularity due to the potential protective effects as a prebiotic to maintain a healthy immune system in infants. According to the WHO, the total world population is estimated to increase by approximately one billion in the next 10 years and reach 9.5 billion by 2050. The health of large populations of newborn infants needs the increasing demand for high-quality nutritional products. According to the estimate of Future Market Insights, the fucosyllactose segment is estimated to occupy a market share of around 48% in terms of value among the various product type segments and sialyllactose with a value share of 28% by 2017 end. The fucosyllactose product type segment is reported to be valued at more than $9 million by the end of 2017 and is estimated to increase with a CAGR (compound annual growth rate) of 14.4% over the forecast period. On the other hand, the market value of sialyllactose is estimated to more than $5 million by the end of 2017 and is expected to increase with a speed of 15.1% per year and valued at more than $20 million by the end of 2027.

31.5 Safety: Toxicity and Side Effects

The toxicity and side effects of oligosaccharides determine their applications in food. Functional oligosaccharides, which were applied in food, must be tested that there was no side effect to organism. Fructo-oligosaccharides, used as sweetener in food, were tested and have no deleterious effects on diabetic rats. Meanwhile, it acts as probiotic applied in anticancer drugs and also has no gastrointestinal discomfort effect on pediatric patients (Bali et al. 2015). Early in 1995, Food Standards Australia New Zealand (FSANZ) revised Australian Food Standard Code and allowed FOS as dietary fiber to be added into food. Following Australia, Denmark, Norway, and Sweden successively published the same code in 1995, 1998, and 1999. The FOS was registered as GRAS (generally regarded as safe) by the FDA for use as food ingredients in November 2000. In China, according to the standard of food nutrition reinforcement (GB14880–2012), FOS can be used into food including infant formula. Nowadays, almost all countries have no limitation on the additive dose of FOS. And the recommend daily intake of FOS in Europe is 20 g and in China is 10 g.

The safety of GOS was assessed by toxicity research in rats and in human. The male and female rats with repeated oral administration of 2,000 mg/kg/day had no GOS-related changes in clinical signs (body weight, feed intake, organ weight, and urine assessment) (Kobayashi et al. 2009). The clinical safety analysis was carried out, in this study, in 50 healthy adults, and 103 constipated patients were treated with 5 g GOS per day, and there was no significant impaired effect in volunteers. Totally, the effective intake of GOS is 3–10 g/d; the maximum daily limit of it is 0.3–0.4/d. Japan is the first country to authorize GOS as Food for Specified Health Uses (FOSHU). Europe had given a license to GOS as food ingredient. US FDA had ruled that GOS was GRAS and given permission to be sold on the market as diet supplement in 2000 (Torres et al. 2010). In addition, GOS had already been

authenticated as new resource food by the State Food and Drug Administration of China in 2008.

As the research moves along, the health function of inulin was deeply investigated. So implying the inulin into the food or drugs is urgent. The safety of inulin was invested in vivo and clinical test. There was an experiment on young adult male mice; the mice treated with 15% inulin incorporated in the basal diet were started 4 weeks before intramuscular transplantation of TLT tumor cells into of the NMRI strain and were continued until the end of the experiment. And they found that dietary treatment with inulin clearly inhibited the growth of a transplantable mouse liver tumor (TLT) and the inulin was nontoxic (Akkerman et al. 2019). Closa-Monasterolo aimed to test the efficacy, safety, and tolerance of Orafti®Synergy1 (oligofructose-enriched inulin) with the dose of 0.8 g/dL on the 4-month-old baby, which was supplemented with infant formula. And they found it is safe and effective with the dose of 0.8 g/dL SYN1-supplemented infant formula for infant and the gut microbiota is closer to that of breastfeeding (Closa-Monasterolo et al. 2013). International Scientific Association for Probiotics and Prebiotics (ISAPP) declared that the inulin was the only prebiotic which was widely accepted by the society in 2016. According to the Europe Union (EU) Commission Regulation 2015/2314 of 7 December 2015, chicory inulin was able to maintain the normal function of gut. What's more, the America Food and Drug Administration (FDA) affirmed that the inulin was generally regarded as safe (GRAS) in 2000 and regarded it as the dietary label in 2016. But recently, Vijay-Kumar et al. found that the mice with dysregulated microbial suffered from liver cancer when fed with inulin. It's no doubt that the latest investigation will subvert the fundamental conception of inulin and other soluble fibers. And it needs relative scientists and organisms make joint effort to explore the safety of inulin.

COS as the functional oligosaccharide was able to be absorbed by intestinal epithelia and distributed to the liver, spleen, and kidney (Kean and Thanou 2010). What's more, the liver plays a crucial role in the degradation of COS (Muanprasat and Chatsudthipong 2017). The mutagenicity, cytotoxicity, and systemic toxicity of COS were evaluated by using in vivo and in vitro models. The experiment on mice with the oral administration for 5 g/kg/d for 180 days showed that the COS has no mutagenicity. And COS has no toxicity on mouse fibroblasts and human lymphocytes. Besides, COS at dose reached for 10 g/kg did not induce the abnormal behavior and has no lethal effect on mice. As for the toxicity of SBOS, there were few experiments to identify the edibility safety and only several toxicological tests toward certain brand products in animals. COS was the preferred functional health product to keep fitness and prevent disease and aging in Japan. Besides, it is the only product which is authorized by the Japanese government for its therapeutic effect. After that, European Community and US FDA had ratified to full product.

As described above, LNnT as a chemically synthesized HMO was applied into infant formula, and its safety was tested through genotoxicity experiment in rats. The study showed that the oral dose reached 5000 mg/kg bw/day on both male and female rats more than 90 days and has no adverse effect by detecting the body weight, fodder consumption, organ weight, and histopathology. What's more, LNnT

was better tolerated compared with fructo-oligosaccharides (Coulet et al. 2013). At the same time, the toxicity of 2'-O-fucosyllactose (2'-FL) was also detected through in vitro assay. And there was no observed adverse effect level with the oral dose for 5,000 mg/kg bw/day on both male and female rats. These findings support the safety of LNnT and 2'-FL to be used in infant formula. European Commission had given permission to use 2'-FL and LNnt into food as additives in 11 March 2016. Two months later, US FDA published same approval. China had solicited public opinion about the use of 2'-FL as food ingredients in 2016.

Nutrition and Allergies (NDA) was asked to have a comment on 2'-FL and LNnT as a novel food ingredient at a request of European Commission, the EFSA Panel on Dietetic Products. After comprehensive consideration on the comments and objections of a scientific nature raised by member states, NDA permitted 2'-FL and LNnT to be used in infant formula foods for specific medical objectives for infants and young children and other foods for infants, young children, and adults (Regulation (EC) No 258/97). This major policy decision took account on a sub-chronic 90-day toxicity study in rat, in which the study found the rats with a dose of 2,000 mg/kg body weight per day had no observed adverse effect. Then applicant conducted a double-blind, random, controlled clinical experiment on infants (before the age of one) orally with 1.2 g/L of 2'-FL and 0.6 g/L LNnT. The 2'-FL and LNnT at a ratio of 2:1 in formulae is safe for infant. The Panel also observed that 2'-FL is nontoxic when combined with other foods at use levels (Coulet et al. 2013).

31.6 Marketed Product

With regard to the function of fructo-oligosaccharides described above, many products including fructo-oligosaccharides were designed in market aiming to improve intestinal tract function and to prompt bowel movement. The form of products is mainly powder. What's more, the products could be used in different age groups. For baby and old, it can be added to the milk powder to enhance the gut health. For adult, it can be mixed with fruit juice to reach the aim of losing weight.

Nowadays, because GOS structurally resembles HMO, it is comprehensively applied in infant formula as functional alternatives to mimic the function of HMO (Moossavi et al. 2018). What's more, the sialylated GOS is also used in infant formula (Wang et al. 2015). Recently, a powdered GOS (Bimuno) product was produced by Clasado Ltd., which was composed of 52% GOS and syrup to modulate the gut stability (Rastall 2010). Inulin as the soluble fiber can regulate intestinal homeostasis and was applied into the food and pharmaceutical. In addition, some weight-losing products were added to inulin with the aim to increase satiety and reduce caloric intake of people. Some infant formulas try to mimic the beneficial effect of human milk using inulin supplementation, mainly because the intestine of newborn is rapidly colonized with microbes and the foods could affect this process.

As mentioned above, the chitin oligosaccharides have the potential to enhance the growth of *Lactobacillus*. So it is made into pressed candy together with the natto to improve the immunity and gut health of organism. Also, there are many brands to

sell the chitosan oligosaccharides in the shape of powder or capsule in the market all over the world. Besides, the chitin oligosaccharides were applied into toothpaste to prevent caries and bad breath. On account of the bioactive physical properties, COS was applied to the potential therapeutic drug, especially the anticancer drug. Recently, Micelle-forming polymeric and chemically modified COS (polycaprolactone-g-COS micelles or ferrocene-modified COS) have been developed as drug carrier and applied to improve drug absorption through epithelial barriers. In recent market, the commercial product of SBOS has well received the attention of customers, such as product manufactured by Calpis Food Industry Company, which is a concentrated syrup, containing 35% SBOSs and solid (Rastall 2010).

The public in the fierce competition must work harder to stay competitive, along with the enormous pressure. Recently, Panasevic created a new formula to ease the pressure of nervous person. The formula contained at least one of HMOs, such as 3′-sialic acid lactose, 6′-sialic acid lactose, lactoyl-*N*-tetrose, and 3′-fucosolactose, which can effectively relieve the pressure. There were various kinds of infant formula milk powder designed for different age group which were sold in the market, such as the high-calcium milk powder and anti-lactoprotein allergy milk powder produced from cow, goat, and donkey milk (Di Renzo et al. 2013). With the aim to provide the most desirable milk powder for infants whose mother could not secrete enough milk, there were part of milk powder which was loaded with human milk oligosaccharides. Abbott Laboratories of the United States firstly launched the HMO formula milk powder in 2016; the additive amount of HMO (2'-FL) is 20 mg/100 mL to mimic breast milk. The HMO formula milk jumped into popularity since it was launched. Now, millions of babies in America have enjoyed the benefits coming from HMO. After that, Wyeth Nutritionals Ireland Company also published 2'-FL formula milk powder with the content of 25 mg/100 mL in 2017. While in 2018, Nestlé company heavily launched the supreme HMO infant milk powder which contained two human milk oligosaccharides (2'-FL and LNnT), and the ratio of 2'-FL and LNnT is 2:1 (100 mg/100 mL, 50 mg/100 mL). The total content of these two oligosaccharides is pretty close to that in breast milk (50–150 mg/100 mL).

31.7 Patents

There were approximately 443 oligosaccharides patents which were retrieved using the oligosaccharide as key word via MedLink online, and there were 195 patents invented in China, 77 patents invented in the United States, 60 patents invented in Korea, 48 patents invented in Japan, 23 patents invented in Europe, and 8 in Germany, and the 24 patents left were published in world class (Fig. 3). What's more, Japan is the earliest country to invent the patents associated with oligosaccharide. The majority of published patents are associated with the manufacture method and application of oligosaccharide. Juan et al. have used the brown algae as the material to produce the brown algae oligosaccharides through enzymolysis approach; in this way, the nondigestible and high-viscosity polysaccharides in kelp were degraded into some low-weight bioactive substance to increase the utilization

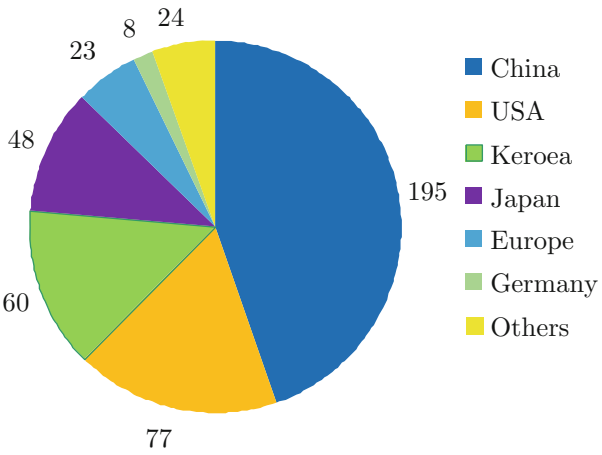

Fig. 3 The number of patents about oligosaccharides in each country

of brown algae. Li et al. have applied for a patent about the craftsmanship and application of core fucosylation oligosaccharide. The feature of this oligosaccharide was the enhanced effect for the growth of *Bifidobacterium* and *Lactobacillus*, which can regulate the intestinal microbial to improve immune function.

31.8 Perspectives

Various functions of oligosaccharides lead to the large-scale production and diverse commercial application in nutraceutical, beverage, infant formula, bread, cheese, and manufactured meat. And there needs more relative research on developing more oligosaccharides with high-function properties to replace antibiotics which can bring side effect to human body. Also translation from the laboratory to clinic faces a large of obstacle, which needs the joint effort of scientists and government. The level of development and research in each country all over the world is uneven, which may indirectly reflect the public demand and cognition of functional oligosaccharide. The health benefits of oligosaccharides are known in varying degrees; there are almost 70% people in Japan who have a deep realization on oligosaccharide, France with 16%, Germany with 9%, and Britain with 3%. So the relevant departments need to strengthen publicity to improve social awareness of functional oligosaccharides. Improving the flavor and nutritional value of nutraceuticals, beverages, and daily food is also an effective method for wider usage in food.

The market for oligosaccharides is already steady and continues to grow gradually. Among the markets, the production levels of some oligosaccharides like FOS and GOS are quite mature and widely deployed. However, the quantity production of the novel oligosaccharides like HMOs still remains in the primary stage. Now, manufacturers of infant formula are using FOS and GOS as affordable substitutes to HMOs because of the low cost and good effect. Hence, these alternatives to human milk oligosaccharides may restrict the application of oligosaccharides in

infant formula to a large scale. Several companies have applied oligosaccharides into food around the world, but applying HMOs into infant formula or other food are costly. However, to decide which compound should be added, the concentrations or combinations of oligosaccharides are needed to correctly determine across abundant tests. Synthetic method is an area which is not fully explored. The output of oligosaccharides can be further increased by efficient separation technique from much more resources. Recently, daily foods and infant foods contain more than one type of oligosaccharide, which encouraged the manufactures and food developers to explore a new and efficient combination of these oligosaccharides used in food. The desired combination of oligosaccharides may help to meet the human body's needs for nutrient and to improve the health value and texture of daily foods.

Growing evidence also clearly demonstrates that the functional oligosaccharides have plenty of physiological function, and part of them are applied in food as nutrient supplements. But in 2018, a new republished article pointed out that the soluble fiber leads mice to liver cancer under the condition of intestinal flora imbalance. Moreover, a mass of clinical trials are expected to confirm the protective effects of the functional oligosaccharide. The public had best abide by dietary guideline in their countries with a proper diet to avoid gut dysfunction. The daily intake dose of oligosaccharides in a safe threshold value is also important. Patents are the sign of inventiveness which can promote the application of oligosaccharides in a larger market. It is necessary to invent more valuable patents in oligosaccharides area to decrease the production cost, increase profits, and range extension application with new technologies and idea. The future of oligosaccharides products seems to be highly promising.

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Saponins in Food

32

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Abstract

Saponins belong to the group of plant glycosides widely distributed in more than 100 families of both wild and cultivated plants and in some marine organisms. They consist of the steroidal or triterpene hydrophobic aglycone and one to three sugar chains (hydrophilic part) attached by ester or ether linkage. Based on number of chains attached to the aglycone, they can be categorized as monodesmosides, bidesmosides, or tridesmosides. To this group of compounds, the glycoalkaloids are also included. Some saponins may contain in their structures (in aglycone part or in sugar chain) glucuronic acid, what makes them acidic. A big number of structurally divergent compounds have been described. Their structures and concertation can be different even in the organs (roots, rhizomes, stems, bark, leaves, seeds, and fruits) of the same plant species. Depending on the structure, they express different biological activities. Triterpenoid saponins can be found in many legumes (alfalfa, soybean, chickpeas, beans, peanuts, broad beans, kidney beans, and lentils), ginseng roots, sunflower seeds, horse chestnut, liquorice roots, spinach leaves, tea leaves, quillaja bark, quinoa seeds, sugar beet, or alliums species. Steroidal saponins can be found in oats, *Yucca*, tomato seeds, yam, fenugreek seeds, ginseng roots, asparagus, aubergine, or capsicum peppers. Glycoalkaloids are characteristic compounds mostly for Soalanaceae species. In general these compounds show no or little toxicity and do not seem to be of hazard for consumers. Depending on the structure, they express different biological activities. In general, saponins have been related to immunostimulatory, hypocholesterolemic, antitumor, anti-inflammatory, antibacterial, antiviral, antifungal, and antiparasitic activities. The major feature of these compounds is their sterol affinity, which seem to be responsible for most of activities they express. When consumed they may provide different health benefits, out of which cholesterol reducing activity is most pronounced.

Keywords

Steroidal saponin · Triterpene saponins · Glycoalkaloids · Sterol affinity · Cholesterolemic activity

32.1 Introduction

Saponins are the group of naturally occurring glycosides which predominate mainly but not exclusively (lower marine animals), in the plant kingdom. They can be found in different plant parts including roots, shoots, flowers, and seeds. The common feature of most of the saponins is the formation of soapy lather when shaken in water solution. In earlier work, saponin distributions in plants were based on this test. In Orient, saponins were used in traditional folk medicine or as a soap, and thus, in many cases, common names of saponin rich plant species are derived from this feature, e.g., soapwort (*Saponaria officinalis*), soaproot

(*Chlorogalum pomeridianum*), soapbark (*Quillaja saponaria*), soapberry (*Sapindus saponaria*), and soapnut (*Sapindus mukurossi*) (Hostettmann and Marston 1995). Some of them find a commercial application as drugs and medicines, adjuvants, taste modifiers, emulsifiers, precursors of hormone synthesis, and sweeteners.

Saponins are complex molecules consisting of nonsugar aglycone coupled to sugar chain units. These sugars can be attached as one, two, or three sugar chains, and hence, the terms of monodesmoside, bidesmoside, and tridesmoside have been given to these saponins, respectively (Greek desmos = chain). The saponins can be divided into two groups containing triterpene or steroidal aglycones – sapogenins (Fig. 1). Some authors also include within the saponins steroidal glycoalkaloids of solanidans and spirosolan classes (Fig. 2). Both triterpene and steroidal aglycones may have a number of functional groups (-OH, -COOH, $-CH_3$). Thus, the number of functional groups and different possibilities of sugar chain composition and attachment causes great natural diversity of saponin structures. Even within one plant species, different parts may harbor saponins with different structures.

Saponins are widely distributed in plant species, being reported in nearly 100 families. They can be found in plants used as human foods such as soya, beans, peas, oat, *Solanum* and *Allium* species, tomato, asparagus, tea, peanut, spinach, sugar beet, yam, and blackberry; in plants used as animal feeding stuffs including alfalfa, clover, legumes, forage and cover crops, sunflower, horse chestnut, guar, and lupine; and in plants used as flavorings, herbs, edible seeds,

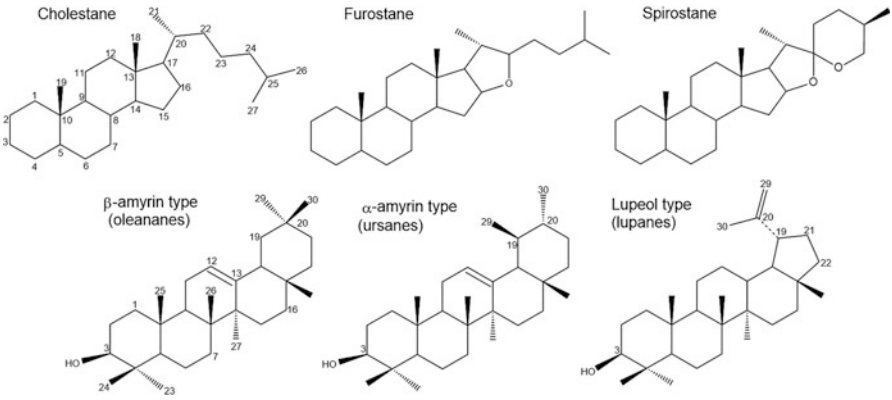


Fig. 1 Sapogenin skeletons – steroidal (upper raw), triterpene (lower raw)

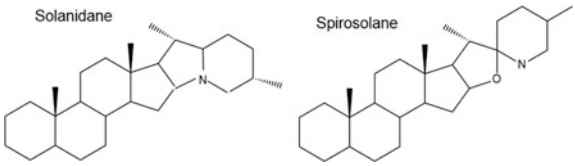


Fig. 2 Glycoalkaloid skeletons

health foods, tonics, etc. including fenugreek, liquorice, nutmeg, *Quillaya*, *Yucca*, *Gypsophila*, and ginseng. Their concentration in plants ranges from traces up to 10% of dry matter, as can be found in *Yucca schidigera* trunk. The concentration depends on the cultivar, plant age, physiological stage, and geographical location. There can be also considerable qualitative and quantitative variation among plant parts.

32.2 Bioactive Constituents of Food Products

32.2.1 Soybean

Soybean has been the world's largest source of protein for human and animal nutrition. Its global production reached about 348 million tons in 2016/2017 season. The key producers are the USA, Brazil, and Argentina, who collectively contribute to 81% to the global soybean production. Soybean seeds, besides having high protein content, contain some nutritionally important natural products such as isoflavonoids, vitamins, and fatty acids. Among these are also oleane-triterpene saponins. Early work on the structures of these compounds provided evidence that soybean saponins are composed of glycosides of three sapogenols, namely, soyasapogenol A (soyasaponins A1 and A2 (Fig. 3)), soyasapogenol B (soyasaponins I, II, III, IV, V, and VI) (Fig. 4), and soyasapogenol E glycosides (Kitagawa et al. 1982, 1984).

R1	R2	Soyasapogenol A
Glc (1-2)-Gal (1-2)-GlcA-	Glc (1-2)-Ara-	Soyasaponin A1
Gal (1-2)-GlcA-	Glc (1-2)-Ara-	Soyasaponin A2

Under acid hydrolysis, some additional soyasapogenols C, D, F, and G were also identified, but they were recognized as artifacts arising from soyasapogenol B (Jurzysta 1984). Later work of Shiraiwa and co-workers (1991a; b) introduced new nomenclature to the soyasaponins. The basic principles of this nomenclature is based on the recognition of only two aglycones, which are soyasapogenol A

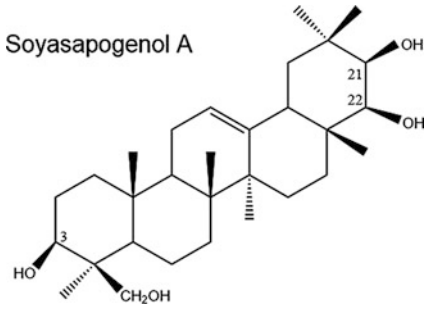


Fig. 3 Soyasapogenol A-derived saponins

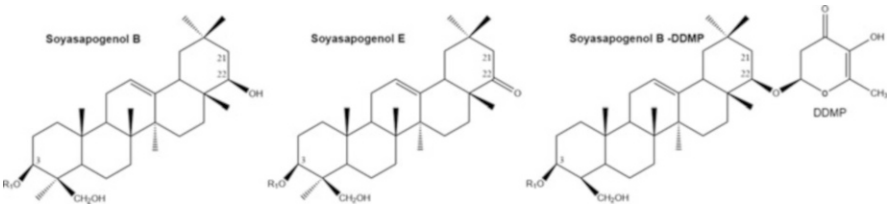


Fig. 4 Soyasapogenol B and E glycosides identified in soybean (soyasaponins according to Kitagawa et al. (1984) and names in parenthesis according to Tsukamoto and Yoshiki (2006))

(group A saponins) and DDMP (DDMP saponins). DDMP in this case was characterized as 2,3-dihydro-2,5-dihydroxy-6-methyl-4Hpyran-4-one(DDMP)-conjugated soyasapogenol B (Fig. 3). The DDMP saponins degrade easily to soyasapogenol B and E glycosides, which take place during separation and workup process. Similar γ-pyron substituted saponins (with trivial name chromosaponin I or soyasaponin VI) were identified in etiolated pea seedlings (*Pisum sativum*), mung bean (*Vigna mungo*), cowpea (*Vigna sinensis*), and alfalfa seeds.

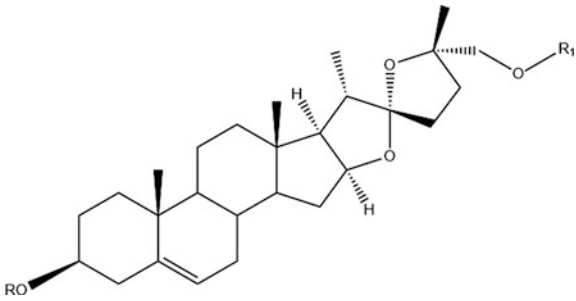
Soyasapogenol A can be substituted with two sugar chains at the C-3 and C-22, and a number of different substitution combinations were identified. Substitutions at C-3 may include -GlcUA-Gal-Glc, -GlcUA-Gal-Rha, -GlcUA-Gal, -GlcUA-Ara-Glc, -GlcUA-Ara-Rha, and -GlcUA-Ara; the C-22 sugars include -Ara-Glc, Ara-Xyl, and Ara. The terminal sugars at C-22 can be fully or partially acetylated, which shows big structural diversity of this group of saponins. Tsukamoto and Yoshiki (2006) reported 50 structurally different saponins, while Chitisankul and co-workers (2018), using ultrafast chromatography system LC–PDA–MS/MS, were able to detect 105 structurally divergent saponins in hypocotyls and cotyledons of nine soybean varieties.

R1	Soyasapogenol B	Soyasapogenol E		Soyasapogenol B-DDMP
Rha(1-2)-Gal(1-2)-GlcA-	Soyasaponin I	(Bb)	(Be)	(βg)
Rha(1-2)-Ara(1-2)-GlcA-	Soyasaponin II	(Bc)	(Bg)	(βa)
Gal(1-2)-GlcA-	Soyasaponin III	(Bb′)	(Be′)	(γg)
Glc(1-2)-Gal(1-2)-GlcA-	Soyasaponin IV	(Ba)	(Bd)	(αg)
Ara(1-2)-GlcA-	Soyasaponin V	(Bc′)	(Bg′)	(γa)
Glc(1-2)-Ara(1-2)-GlcA-	Soyasaponin VI	(Bx)	(Bf)	(αa)

32.2.2 Oats

Oats (*Avena sativa* L.) are very important source of livestock feed worldwide as nutritious grain or as forage. This is the grain crop with highest protein, fiber, and mineral content as compared to other grains. Its production worldwide decreased dramatically during last 50 years, but at present, it constitutes about 2% of global

Fig. 5 Oat saponins



grain production, but in some countries, this accounts for 5%. Oats are also used in the production of human food products, e.g., oatmeal, oat flower, oat bran, and oat flakes. These products are used for breakfast cereals and as ready-to-eat oat products. They can be included in gluten-free diets.

Chemically, oat hulled seeds are good source of water-soluble fiber, antioxidants, tocols, phenolic acids, aventhramides, flavonoids, and sterols. They also contain steroidal saponins. This is worth to notice that *A. sativa* is a unique plant species, which contains two types of saponins, steroidal saponins (avenacosides) in aerial parts and triterpene saponins (avenacins) in roots. The grain contains four saponin glycosides, including avenacoside A, avenacoside B, desglucoavenacoside A, and desglucoavenacoside B (Fig. 5). Very recently it was documented that one of the glucoses is sulfated, and the compounds were identified as nuatigenin 3-*O*-{α-L-rhamnopyranosyl-(1-2)-[β-D-6-*O*-sulfoglucopyranosyl-(Barakat et al. 2015; Böttcher and Drusch 2017; Bushway et al. 1994; Chitisankul et al. 2018)]-β-D-glucopyranoside}-26-*O*-β-D-glucopyranoside (Pecio et al. 2012). These sulfo-derivatives occurred in trace amounts. The average total concertation of saponins in oats grain as determined for sixteen varieties was 685 μg/g, while in oat husks, it was 25 μg/g of dry matter (Önning et al. 1993; Pecio et al. 2013).

Glycoside	R	R_1
Avenacoside A	Glc(1-4)[Rha(1-2)]Glc-	Glc
Avenacoside B	Glc(1-3)-Glc(1-4)[Rha(1-2)]Glc-	Glc
26-Desglucoavenocoside A	Glc(1-4)[Rha(1-2)]Glc-	H
26-Desglucoavenacoside B	Glc(1-3)-Glc(1-4)[Rha(1-2)]Glc-	H

32.2.3 Alfalfa Seedlings

The human consumption of alfalfa products is generally low, but in some countries, especially in Orient, alfalfa sprouts are being used as a green salad. Sprouts are usually added to soups, on top of sandwiches, and to salads. It is estimated that 100 g of sprouts contain 23 cal, 2.1 g carbohydrates, 3.99 g protein, 0.69 g fat, 1.9 g fiber, 30.5 μg vitamin K, 8.2 mg vitamin C, 36 μg folate, 0.2 mg manganese, 0.2 mg

copper, 70 mg phosphorus, 27 mg magnesium, 0.1 mg riboflavin, 0.9 mg zinc, 1 mg iron, 0.1 mg thiamine, and 155 IU vitamin A.

But alfalfa contains also saponins, which at least in relation to animal nutrition, are recognized as antinutritional compounds. Early studies on alfalfa seedlings showed that they can be extremely rich with saponins (8–10% of DM) due to rapid synthesis of biologically active saponins in germinating seeds and sprouts. Reinvestigation of the germination process performed with analytical HPLC procedure provided completely different picture of saponin synthesis in alfalfa seedling (Oleszek 1998). It was documented that for the first 3 days of germination process, only soyasaponin I can be detected at the level of 2.12 μmol/g, the same as in genuine seeds. On the 4th day of germination, the synthesis of medicagenic acid glycosides started. The first compound of this group was monodesmosidic 3-*O*-glucoside of the medicagenic acid. Its concentration was increasing gradually and established at the level of 1.2–1.3 μmol/g after 11th day of germination. On the 5th day, bidesmosidic 3GlcA,28AraRhaXyl medicagenic acid and on the 6th day 3Glc,28Glc medicagenate were observed. Their concentrations ranged between 0.82% and 1.8% DM and between 0.06 and 1.04 μmol/g, respectively, and after 10 days showed quite stable levels. Zahnic acid tridesmoside (3GlcGlcGlc,23Ara,28AraRhaXylApi 16-OH medicagenate), one of the dominant compounds of alfalfa tops, appeared for the first time after 12 days of seedling growth at the concentration, which was comparable to that of medicagenic acid glycosides.

R	R1	
Medicagenic acid		
Glc-	H	Roots
Glc-	Glc-	Roots
Glc-(1-4)-Glc-	H	Roots
Glc-(1-6)-Glc-(1-3)-Glc-	H	Roots
Glc-(1-6)-Glc-(1-3)-Glc-	Glc-	Roots
Glc-	Xyl-(1-4)-Rha-(1-2)-Ara-	Roots, tops
Glc-(1-2)-Glc-(1-2)-Glc-	Xyl-(1-4)-Rha-(1-2)-Ara-	Roots
Glc-(1-2)-Glc-(1-2)-Glc-	Xyl-(1-4)-[Api-(1-3)]- Rha-(1-2)-Ara-	Roots
Glc-(1-2)-Glc-	Xyl-(1-4)-Rha-(1-2)-Ara-	Roots, tops
Glc-	Rha-(1-2)-Ara-	Roots
GlcUA-	H	Roots
GlcUA-	Xyl-(1-4)-Rha-(1-2)-Ara-	Roots, tops
GlcUA-	Rha-(1-2)-Ara-	Roots
Gal-(1-2)-Glc-	Glc	Roots
Rha-(1-2)-Glc-(1-2)-Glc-	Glc	Roots
H	Xyl-(1-4)-Rha-(1-2)-Ara-	Roots
Hederagenin		
Glc-(1-2)-Ara-	H	Roots
Ara-(1-2)-Glc-(1-2)-Ara-	H	Roots
Ara-(1-2)-Glc-(1-2)-Ara-	Glc-	Roots

(continued)

R	R1	
Gal-(1-2)-Ara-	H	Roots
Zanhic acid		
Glc-(1-2)-Glc-(1-2)-Glc-	Xyl-(1-4)-[Api-(1-3)]- Rha-(1-2)-Ara-	Roots tops
Glc-(1-2)-Glc-(1-2)-Glc-	Xyl-(1-4)-Rha-(1-2)-Ara-	Roots

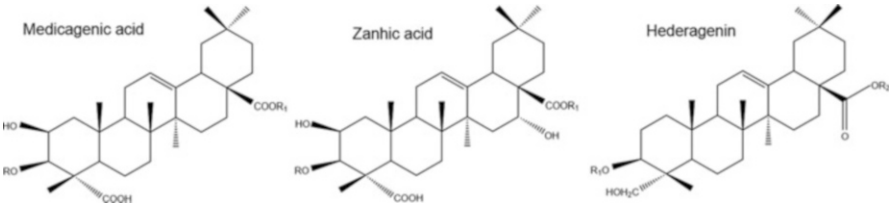


Fig. 6 Alfalfa saponins

Thus, 2-week-old seedlings presumably contain full spectrum of alfalfa root and top glycosides of medicagenic acid, zanhic acid, and hederagenin (Fig. 6). Additionally, soyasaponin I (for structure see section soybean) is also one of major components of roots and tops. The consumption of the sprouts as a garnish should not create health hazards. However, this should be kept in mind that medicagenic acid glycosides are hemolytic. They bind intestine membranes, increasing their permeability, while zanhic acid glycosides show throat irritating properties (Oleszek et al. 1992, 1994).

32.2.4 Beans and Peas

The worldwide production of bean and pea during last few years ranges around 22.5 and 13 million metric tons, respectively. They are recognized as a rich protein source in human and animal nutrition. The incorporation of legume ingredients into commonly consumed food products represents a viable alternative for complementing the protein deficiency in developing countries. Legume-based ingredients are being used to develop breads as a potential means of decreasing the risk of cardiovascular disease (Barakat et al. 2015).

All of the grain legumes possess saponins as a chemical ingredient, usually the group B saponins (Bb, Be, and βg). Soyasaponin I (Bb) has been found in numerous legumes, including kidney bean (*Phaseolus vulgaris*), runner bean (*P. aureus*), butter bean (*P. lunatus*), scarlet runner bean (*P. coccineus*), field bean (*Vicia faba*), lentil (*Lens culinaris*), and pea (*Pisum sativum*). Chickpeas

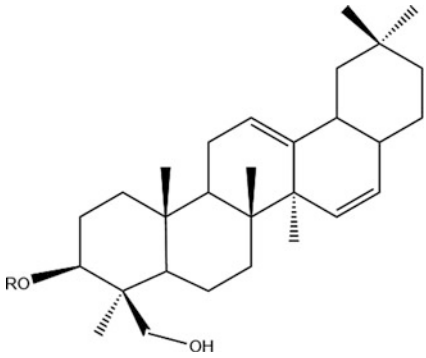


Fig. 7 Phaseollosides D and E

(*Cicer arietinum*) contain mainly soyasaponin βg and lower amounts of Bb and Be (Serventi et al. 2013).

Kidney bean (*Phaseolus vulgaris*) contains additionally glycosides of ursa-12,15-dien-3,24-diol, so-called phaseollosides A to E. Two major glycosides, phaseollosides D and E, are presented in Fig. 7.

Glycoside		R
Phaseolloside	D	Rha(1-6)[Gal(1-2)]Glc(1-4)Gal(1-2)Ara(1-3)GlcA-
Phaseolloside	E	Glc(1-4)Glc(1-4)Gal(1-2)[Rha(1-6)]Glc(1-4)Gal(1-2)Ara(1-3)GlcA-

The adzuki bean (*Vigna angularis*), known also as red mung bean, is widely consumed throughout East Asia. It contains, besides a high nutrient content, also the highest fiber content of all other beans. It contains also saponins which are glycosides of soyasapogenol B (azukisaponin II and V), sophoradiol (azukisaponin I), azukisapogenol (azukisaponin III and VI), and gypsogenic acid (azukisaponin IV) (Kitagawa et al. 1983a, b) (Fig. 8).

Glycoside	R1	R2	R3	R4	R5
Azukisaponin I	Glc(1-2)GlcA-	-H	-CH3	-OH	-CH3
Azukisaponin II	Glc(1-2)GlcA-	-OH	-CH3	-OH	-CH3
Azukisaponin III	Glc(1-2)GlcA-	-OH	-COOH	-H	-CH3
Azukisaponin IV	Glc-	-COOH	-CH3	-H	-COO-Glc(6-1)Glc
Azukisaponin V	Rha(1-2)Glc(1-2)GlcA-	-OH	-CH3	-OH	-CH3
Azukisaponin VI	Glc(1-2)GlcA-	-OH	-COO-Glc(6-1)Glc	-H	-CH3

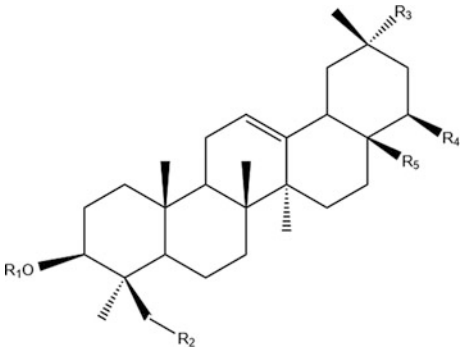


Fig. 8 Adzuki bean saponins

32.2.5 *Allium* Species

The genus *Allium* contains about 800 species distributed all over the world. They all contain saponins as natural products with different spirostanol, furostanol, and cholestane aglycones. Out of this huge number of species, three are important food products. This includes onions (*Allium cepa* L.), leek (*Allium porrum* L.), and garlic (*Allium sativum* L.).

Onions contain a number of saponins of furostanol and spirostanol nature with most dominant aglycones, namely, diosgenin (25*R*)-spirost-5(6)-ene-3β-ol), (25*S*), ruscogenin (25*S*)-spirost-5(6)-ene-1β,3β-diol), and cepagenin (24*S*,25*R*)-spirost-5(6)-ene-1β,3β,24-triol). The identified structures are listed in Table 1.

Leek contains glycosides of the following aglycones: diosgenin, gitogenin [(25*R*)-5a-spirostane-2α,3β-diol], β-chlorogenin [(25*R*)-5α-spirostane-3β,6β-diol], porrigenin B [(25*R*)-5α-spirostane-3β,6β-diol-2-one], neoporrigenin B [(25*S*)-5-α-spirostane-3β,6β-diol-2-one], 12-ketoporrigenin [(25*R*)-5α-spirostane-3β,6β-diol-12-one], porrigenin C [(25*R*)-5α-spirostane-3β,6β-diol-2,12-dione], agigenin [(25*R*)-5α-spirostane-2α,3β,6β-triol], neoagigenin [(25*S*)-5α-spirostane-2α,3β,6-β-triol], porrigenin A [(25*R*)-5α-spirostane-2β,3β,6β-triol], neoporrigenin A [(25*S*)-5α-spirostane-2β,3β,6β-triol], and 2,3-seco-porrigenin [(25*R*)-5-α-2,3-secospirostane-2,3-dioic acid-6β-hydroxy-3,6-c-lactone] (Lanzotti 2005).

Garlic contains furostanol saponins occurring as pairs of inseparable HO-22α and HO-22β isomers. These include voghieroside A1/A2 and voghieroside B1/B2, having agapanthagenin as an aglycone; voghieroside C1/C2, possessing agigenin aglycone; and voghieroside D1/D2 and E1/E2, with gitogenin as an aglycone (Lanzotti 2012).

It possess also two spirostanol saponins including agigenin 3-*O*-trisaccharide and gitogenin 3-*O*-tetrasaccharide. In some other varieties additionally sativiside-R2, F-gitonin, dideglucoerubo side B, iso-eruboside B, sativoside B1, proto-eruboside B, and proto-iso-eruboside B were also identified (Sobolewska et al. 2016) (Table 1)

Table 1 Saponins contained in *Allium* species

Glycoside	Sapogenin	Sugar residue
Onion (*Allium cepa*)		
	Diosgenin	3-Rha(1-2)Ara
	Diosgenin	3-Gal-(1-4)Rha(1-2)Ara
	Diosgenin	3-Glc(1-2)[Glc(1-3)]Gal(1-4)Rha (1-2)Ara
Alliospiroside A	(25S)-ruscogenin	1-Rha-(1-2)Ara
Alliospiroside B	(25S)-ruscogenin	1-Rha-(1-2)Gal
Alliospiroside C	Cepagenin	1-Rha-(1-2)Ara
Alliospiroside D	Cepagenin	1-Rha-(1-2)Gal
Alliofuroside A	(25S)-furost-5(6)-ene-1b,3β,22α,26-tetrol	1-Rha-(1-2)Ara, 26-Glc
Ceparoside A	(25R)-22-methoxy-furost-5(6)-ene-1β,3β,22α,26-tetrol	1-Rha-(1-2)Ara, 26-Glc
Ceparoside B	(25R)-furost-5(6)-ene-1β,3β,22α,26-tetrol	1-Rha-(1-2)Ara, 26-Glc
Ceparoside C	(25R)-furost-5(6),20(22)-diene-3β,26-diol	3-Glc(1-4)[Rha(1-2)]Gal, 26-Glc
Ceparoside D	(25S)-furost-5(6),20(22)-diene-3β,26-diol	3-Glc(1-4)[Rha(1-2)]Gal, 26-Glc
Leek (*Allium porrum*)		
	Gitogenin	3-Glc(1-3)Glc-(1-2)-[Xyl(1-3)]Glc (1-4)Gal
	β-Chlorogenin	3-Glc(1-2)[Glc(1-3)]Gal, 6-Glc
	β-Chlorogenin	3-Glc(1-2)[Xyl(1-3)]Glc(1-4)Gal
	β-Chlorogenin	3-Glc(1-3)Glc(1-2)Xyl(1-3)]Glc (1-4)Gal
	12-Ketoporrigenin	3-Glc(1-2)[Xyl(1-3)]Glc(1-4)Gal
Porrigenin B		3-Glc(1-3)Glc(1-2)-[Glc(1-3)]Glc (1-4)Gal
Porrigenin C		3-Glc(1-2)[Xyl(1-3)]Glc(1-4)Gal
	Alliosterol	1-Rha, 16-Glc
	Alliosterol	1-Glc(1-4)Rha, 16-Gal
Garlic (*Allium sativum*)		
Sativisode-R2	Tigogenin	3-Glc(1-3)Glc(1-2)[Xyl(1-3)]Glc (1-4)Gal
F-Gitonin	β-Chlorogenin	3-Gal
Dideglucoerubo side B	β-Chlorogenin	3-Glc(1-4)Gal
Iso-eruboside B	(25S)-5α-spirostane-3β,6β-diol	3-Glc(1-2)[Glc(1-3)]Glc(1-4)Gal
Sativoside B1	(25R)-5α-furostane-3β,6β,22,26-tetrol	3-Glc(1-3)Glc(1-2)[Glc(1-3)]Glc (1-4)Gal, 26-Glc
Sativioside R1	(25R)-5α-furostane-3β,22,26-triol	3-Glc(1-3)Glc(1-2)[Xyl(1-3)]Glc (1-4)Gal, 26-Glc

(continued)

Table 1 (continued)

Glycoside	Sapogenin	Sugar residue
Proto-eruboside B	(25R)-5α-furostane-3β,6β,22,26-tetrol	3-Glc(1-2)[Glc(1-3)]Glc(1-4)Gal, 26-Glc
Proto-iso-eruboside B	(25S)-5α-furostane-3β,6β,22,26-tetrol	3-Glc(1-2)[Glc(1-3)]Glc(1-4)Gal, 26-Glc
Voghieroside A1/A2	Furostane-2α,3β,5α,22α(22β),26-pentol	3-Glc(1-3)Glc(1-2)[Glc(1-3)]Glc (1-4)Gal, 26-Glc
Voghieroside B1/B2	Furostane-2α,3β,5α,22α(22β),26-pentol	3-Glc(1-2)[Glc(1-3)]Glc(1-4)Gal, 26-Glc
Voghieroside C1/C2	Furostane-2α,3β,6β,22α(22b)	3-Glc(1-2)[Glc(1-3)]Glc(1-4)Gal, 26-Glc
Voghieroside D1/D2	Furostane-2α,3β,22α(22β),26-tetrol	3-Glc(1-3)Glc(1-2)[Glc(1-3)]Glc (1-4)Gal, 26-Rha
Voghieroside E1/E2	Furostane-2α,3β,22α(22β),26-tetrol	3-Glc(1-2)[Glc(1-3)]Glc(1-4)Gal, 26-Rha

32.2.6 *Asparagus* (*Asparagus officinalis* L.)

Only white young shoots of asparagus are commonly used as a food product. They quickly turn green and woody. White shoots contain 93% of water and are low in calories and sodium, but they contain different vitamins and minerals. White shoots, in contrary to green ones, are bitter, and steroidal saponins are recognized as responsible for this bitterness. Asparagus saponins belong to the group of steroidal glycosides, which can be divided into furostanol and spirostanol types. On the enzyme or microbial activities, Glc from position C26 can be eliminated, as happens in many cases with steroidal saponins, with the formation of spirostan derivatives. Such compounds have been reported in literature as asparagosides A to I. The dominant saponin identified in this species is protodioscin (asparasaponin I) (Kawano et al. 1977; Corinna and Hofmann 2012) (Fig. 9).

Aglycone	Saponin trivial name	R
A: (25S)-5β-furostan-3β,22α,26-triol	Officinalisnin I	Glc(1-2)Glc-
	Officinalisnin II	Xyl(1-4)[Glc(1-2)]Glc-
(25R)-furostane-3β,22,26-triol		Rha(1-4)Glc-
B: (25S)-furost-5-en-3β,22α,26-triol	Asparasaponin I	Rha(1-4)[Rha(1-2)]Glc-
	Asparasaponin II	Rha(1-4)Glc-
(25R)- furost-5-en-3β,22α,26-triol		Rha(1-4)Glc-
(25S)-spirost-5-ene-3β-ol		Rha(1-4)[Rha(1-2)]

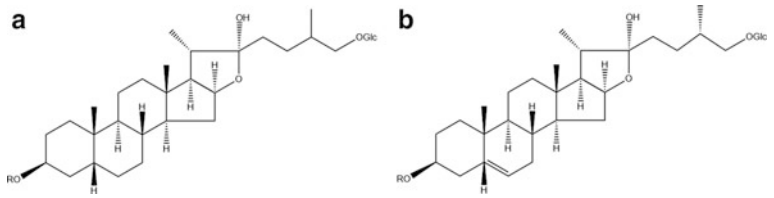


Fig. 9 Steroidal saponins from asparagus: (**a**), sarsapogenin; (**b**), yamogenin

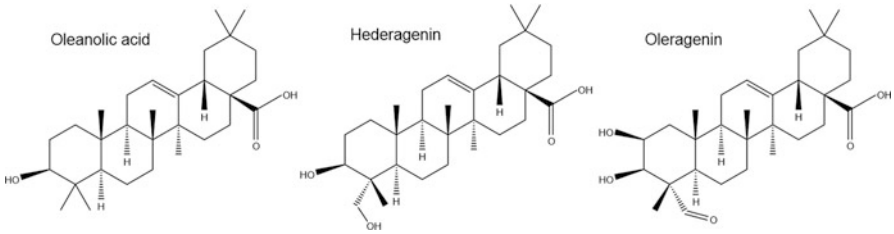


Fig. 10 Aglycones of the spinach saponins

32.2.7 *Spinach* (*Spinacia oleracea* L.)

Spinach leaves are commonly eaten as a vegetable, either fresh, frozen, canned, chipped, or dehydrated. Spinach, along with other green and leafy vegetables, contains an appreciable amount of iron, attaining 21% of the daily value in a 100 g. It also contains saponins, but the work on them is quite limited. An early work on spinach roots showed the presence of two monodesmodisic saponins – spinasaponin A, having oleanolic acids as the aglycone, identified as 3-*O*-[Glc(1-3) GlcA]-12-en-3β-ol-28-oic acid and spinasaponin B having hederagenic as the aglycone, identified as 3-*O*-[Glc(1-3)GlcA]-olean-12-en-3β,23-diol-28-oic acid (Tschesche et al. 1969). Next, a new bidesmosidic pentacyclic triterpenoid saponin, oleragenoside, was isolated from *Spinacia oleracea* L. plants (Mithöfer et al. 1999). Its novel aglycone, representing 2-hydroxy-gypsogenin, was named oleragenin. The structure was established on the basis of spectral data as oleragenin-28-*O*-β-D-glucopyranoside-3-β-*O*-D-glucuronopyranoside. All aglycones of spinach saponins are presented in Fig. 10.

32.2.8 Sugar Beet and Red Beet

The sugar beet root, as a row material for the production of sugar, was reported to contain triterpene saponin, being the glycosides of oleanolic acid (see Spinach saponins section). So far, two saponins were identified which were 3-*O*-GlcA and

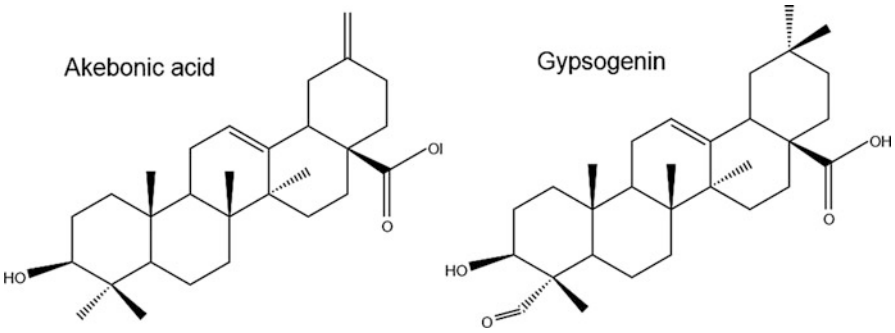


Fig. 11 Aglycones of red beet saponins

3-*O*-Glc of oleanolic acid. Similarly, ten betavulgarosides (I-X) were identified in red beet, with oleanolic acid glycosides as dominant saponins (Mroczek et al. 2012; Yoshikawa et al. 1996). Using hyphenated techniques, e.g., reversed-phase liquid chromatography, combined with negative-ion electrospray ionization quadrupole mass spectrometry, a number of minor saponins were identified in red beet extracts. In total, 44 saponins and 10 groups of isomers were detected and tentatively identified. The identified saponins were glycosides that consist of four different sapogenins, two of which were not previously detected in *B. vulgaris* L. These are akebonoic acid and gipsogenin (Mikołajczyk-Bator and Pawlak 2016) (Fig. 11). But again, the dominant saponins were confirmed to be oleanolic acid glycosides.

32.2.9 Tea Saponins

The total tea consumption of the world is 3,000,000 t a year. The highest consumption is recorded in India, China, and Japan. But, it is a popular drink almost all over the world. Tea leaves contain high amounts of phenolics with antioxidant activities. Nonetheless, they also contain saponins, which vary depending on the parts of plant (leaf, flowers, and seeds). The leaf saponins, that have so far been identified, contain theasapogenol B as an aglycone. Most of them are acylated with cinnamoyl group as a functional group, but acylation with angeloyl, tigloyl, and acetyl groups is also common. At present, ten different glycosides have been identified (Fig. 12).

Trivial name	R0	R1	R2	R3	R4
Theasaponin B1	Xyl(1-2)Ara(1-3)[Gal (1-2)]GlcA-	(E)-Cin	Ac	H	Ac
Assamsaponin J	Ram(1-2)Ara(1-3)[Gal (1-2)]GlcA-	Ac	(E)-Cin H	Ac	
Isotheasaponin B1 (Foliatheasaponin V)	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	(E)-Cin	H	Ac	H
Isotheasaponin B2	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	Ac	(E)-Cin H	H	

(continued)

Trivial name	R0	R1	R2	R3	R4
Isotheasaponin B3	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	Ang	(E)-Cin H	H	
Foliatheasaponin I	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	Tig	Ac	H	Ac
Foliatheasaponin II	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	(E)-Cin	Ac	H	H
Foliatheasaponin III	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	Ang	Ac	H	Ac
Foliatheasaponin IV	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	(Z)-Cin	Ac	H	Ac
Floratheasaponin A	Xyl(1-2)Ara(1-4)[Gal (1-2)]GlcA-	Ang	Ac	H	H

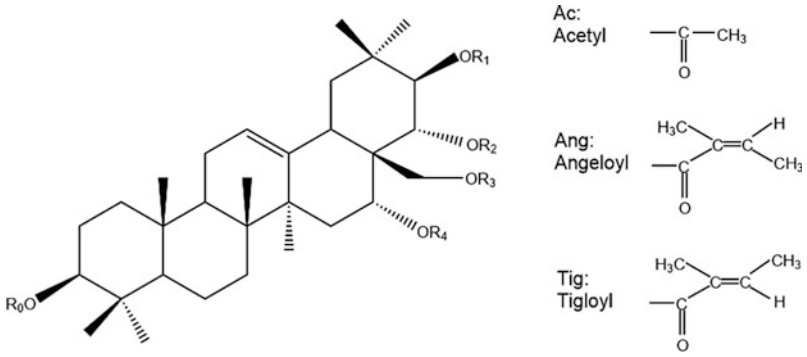


Fig. 12 Tea saponin structures

32.2.10 Potato Glycoalkaloids

Globally, potatoes are the world's fourth largest food crop after maize (corn), wheat, and rice. The tubers contain high amounts of carbohydrates (88% is starch) about 2% of protein and are a rich source of vitamin B6 and vitamin C. Nevertheless, potato aerial parts, as well as tubers, contain glycoalkaloids, which are generally recognized as toxic for consumers. Tubers contain glycoalkaloids, concentrated predominantly in a skin layer, and after peeling, they do not create health hazard. However, when exposed to the light, tubers may accumulate considerable amounts of glycoalkaloids and may be dangerous for health. New varieties of potatoes undergo screening for the glycoakaloid content, as their acceptable concentration in tubers should not be higher than 20 mg/100 g fresh weight. Concentration higher than 20 mg causes bitter taste of potatoes and a burning sensation in the mouth and throat. This should be also

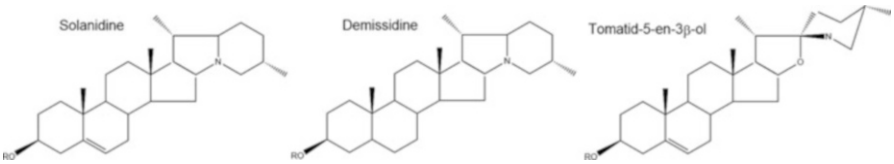


Fig. 13 Glycoalkaloids of potato tubers

mentioned that in animal feeding, tubers, peels, leaves, and sprouts are used. In such a case, care should be taken, as leaves, stems, and sprouts may contain 145, 32–46, and 997 mg/100 g fresh weight, respectively. Glycoalkaloids of the tubers are the mixture of two glycosides of solanidine, α-solanine, and α-chaconine, which are trisaccharides (Fig. 13), but some β and γ forms of these two glycosides, corresponding to di- and monosaccharides, were also detected. Moreover, the aglycone solanidine can also be present in plant tissue. The ratio of solanine to chaconine may widely vary in different varieties. The α-chaconine content may range from 1.7 to 13.5 mg/100 g fresh weight, while solanine values range from 0.58 to 5.9 mg/100 g (Kondamudi et al. 2017). This is an important issue for food safety as chaconine was proved to be more toxic than solanine. The aglycones are substantially less toxic as their glycosides. Nevertheless, generally, orally given potato glycoalkaloids are moderately toxic, because of relatively low gastrointestinal absorption. Some varieties of potato, which were bred for different kinds of resistance, using wild *Solanum* species, such as *Solanum chacoense*, *Solanum demissum*, and *Solanum commersoni*, may contain glycoalkaloids present in these species. Thus, other glycoalkaloids, such as α-solamarine and β-solamarine, demissidine and its glycosides demissine and commersonine (Fig. 12), as well as 3β-solanidan-3α-ol, can be found in new varieties (Friedman 2006).

Aglycone	Trivial name	R
Solanidine	α-chaconine	Rha(1-2)[Rha(1-4)]Glc-
Solanidine	α-solanine	Rha(1-2)[Glc(1-3)]Gal-
Demissidine	Demissine	Glc(1-2)[Xyl(1-3)]Glc(1-4)Gal-
Demissidine	Commersonine	Glc(1-2)[Glc(1-3)]Glc(1-4)Gal-
tomatid-5-en-3β-ol	α-solamarine	Rha(1-2)[Glc(1-3)]Gal-
tomatid-5-en-3β-ol	β-solamarine	Rha(1-2)[Rha(1-4)]Glc-

32.2.11 Tomato

The global production of tomatoes amounts to approximately 160 million tons annually, out of which a quarter is used for industrial processing, including pickled green, green fried, dried red, ketchup, pomace, etc. Nutritional value of these products is rather not high, but they contain antioxidants, carbohydrates, fiber, flavor compounds, minerals, proteins, vitamins, and calistegines. Health-promoting activities of some chemicals, e.g., lycopene, are well documented.

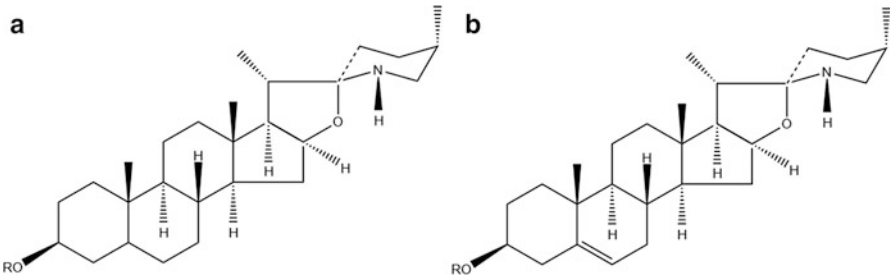


Fig. 14 Tomato saponins structures

However, tomatoes, like numerous solanaceous plant species, contain glycoalkaloids, which are recognized as not desired compounds. Tomatoes contain two glycosides – dehydrotomatine and α-tomatine (Fig. 14). Their concentration strongly depends on the fruit maturity. During ripening process, a sharp decrease in tomatine content is being observed, and fully mature fruits are almost free of these compounds. The tomatine content in green fruits (10 days after flowering) ranged from 500 to 800 μg/g, while the concentration of dehydrotomatine ranged from 40 to 70 μg/g of fresh weight. The ratio of dehydrotomatine to tomatine ranged from 1:9 to 1:12 and was similar in different varieties. In mini-tomato plants, total concentration of tomatines in green fruits was approximately 18,000 μg/g of fresh weight. Fifty days after flowering, total tomatine concentration dropped to nearly zero (1.5–3.7 μg/g of fresh weight) (Kozukue et al. 2004). The concentrations of tomatine in the processed tomato products, such as juice, red sauce, fried green, microwaved green, ketchup, pickled green, tomatillos, and canned tomato sauce, were 28, 57, 11, 12, 25, 28–120, 6, and 64 μg/g, respectively. Tomatine is a biologically active molecule, as shown by its ability to disrupt cell membranes, bind cholesterol, inhibit acetylcholinesterase, and perturb acid-base equilibria in vivo. Like some other saponins, it is also hemolytic and increases the permeability of membranes of the surface of averted rat jejunal sacs. Thus, the increase in gut permeability may be associated with some forms of food allergy.

Aglycone	Trivial name	R
A (25S)-22βN-5α-spirosolan-3β-ol	Tomatidine	H
	α-tomatine	Xyl(1-3){Glc(1-2)]Glc(1-4)Gal
B (25S)-22βN-spirosal-5-en-3β-ol	Tomatidenol	H
	Dehydrotomatine	Xyl(1-3){Glc(1-2)]Glc(1-4)Gal

32.2.12 Concentration of Saponins in Food Plants

The concentration of saponins in different raw plant products (Table 2) ranges for particular species from trace amounts to quite high concentrations. This depends strongly on many parameters, including variety, growth conditions (fertilization,

Table 2 Saponin contents in food and feed plants

Species	Common name	Concentration (% DW)
Allium cepa	Onion	0.02–0.5
Allium porum	Leek	0.10–1.0
Allium sativum	Garlic	0.09–1.5
Asparagus officinalis	Asparagus	1.5
Avena sativa	Oats	0.1–0.2
Beta vulgaris	Red beet	0.7–1.2
Cicer arietinum	Chickpea	0.26–6.0
Glycine max	Soybean	0.5–2.5
Lens culinaris	Lentil	0.1–0.5
Lycopersicon esculentum	Tomato	
	Green	0.03–0.12
	Red	0.00–0.05
Medicago sativa	Alfalfa sprouts	0.6
Phaseolus aureus	Runner bean	0.34
Phaseolus lunatus	Butter bean	0.10
Phaseolus vulgaris	Kidney bean	0.35–1.6
Pisum sativum	Pea	0.01–0.18
Solanum tuberosum	Potato	0.02–0.06
Spinacia oleracea	Spinach	0.07–4.7
Vicia faba	Field bean	0.35
Vigna angularis	Adzuki bean	0.02–0.4
Vigna mungo	Mung bean	0.05–0.57
Vigna sinensis	Cowpea	0.06–0.5

temperature, and water availability, light), the harvest stage, and some post-harvest storage conditions. Very important is also the technique of measurement. In older literature, where concentration was determined with gravimetric, spectrophotometric, or densitometric techniques, these values are usually quite high and should be considered as strongly overestimated. Modern techniques that include liquid chromatography with UV/MS detection provide lower values and are more reliable (Ha et al. 2014).

This should be considered that both steroidal and triterpene saponins are not toxic to humans and animals, or their toxicity is very low. Thus, even concentrations reaching 1–2% should not create any health problems.

Different situation appears in case of glycoalkaloids in potato and green tomato. USDA has defined consumption limits for total glycoalkaloids (TGAs) that should be lower than 200 mg/kg fresh weight (FW) or 1000 mg/kg dry weight (DW) in potato. It is well documented that concentration of α-solanine in potato higher than 140 mg/kg FW caused bitter taste and burning sensation in the throat and mouth. Moreover, in human, the noxious dose of TGA is 2–5 mg/kg body weight (BW), and the fatal dose is more or less 3–6 mg/kg BW. Most commercial potato tubers have been reported to contain between 200 and 600 mg/kg, and 40–120 mg/kg of

glycoalkaloids in freeze-dried material and FW, respectively. Concentrations of glycoalkaloids are typically three to ten times greater in the potato peel than flesh. Thus, peeling potatoes removes most of glycoalkaloids, but care has to be taken for storage of peeled potatoes, as glycoalkaloid concentration may drastically increase during 48 h after peeling. The concentration of glycoalkaloids may also substantially increase during the post-harvest storage of potatoes exposed to intensive light. Greening of tubers under such conditions is accompanied with substantial increase in glycoalkaloid level. Even though literature reports for total glycoalkaloid content in several potato varieties are available, the total glycoalkaloid content in commercial potato products is not reported, and its determination is often complicated by complex constituent mixtures of food ingredients. Thus, this is of great importance to measure glycoalkaloid content in raw material used for processing, as they are resistant to degradation by household or industrial food processing methods, including exposure to boiling water, oven baking, deep frying, and microwave irradiation.

For tomatoes, a range of 49.8–572.5 mg of tomatine per kg of FW of green tomatoes was reported (Bushway et al. 1994). Other sources indicate that dehydrotomatine and α-tomatine content of tomatoes varied from 42 to 1498 and 521 to 16,285 mg/kg of FW, respectively. The ratio of α-tomatine to dehydrotomatine ranged from 10.9 to 12.5 in tomatoes, and from 2.3 to 7.8 in the other plant tissues. This variation can also be explained by variety and developmental stages. Tomatine is almost nonexistent in red tomatoes, because it has been widely demonstrated that tomatine is degraded during ripening.

32.3 Bioactivities, Bioavailability, and Metabolism

Biological activities of saponins are structure dependent. Both in steroidal, as well as between triterpene saponins, there are compounds showing high biological activities and glycosides lacking any proven activity. It seems that the most important factor determining activity is the structure of aglycone, in particular, the number and the location of functional groups. For example, between triterpene saponins, those having soyasapogenols as the aglycone show limited activity, while those having more -OH or COOH groups, e.g., medicagenic acid, hederagenin, oleanolic acid, etc., show substantial biological activity. For those active compounds, the level of activity is strongly dependent on the number and the structure of the sugar chains. Generally, monodesmosides show much higher activities than bi- or tridesmosides (Table 3).

The most important feature of saponins, determining their activity, is the sterol affinity of some of them. Saponin molecule possesses hydrophobic aglycone and very polar sugar chain. Hydrophobic part may react with sterols of membranes, changing their permeability. When saponin binds the erythrocyte cell membrane, it causes membrane disruption and lysis of the cell. Such hemolytic effect eliminates some saponins to be used as pharmaceutical or as food additive.

Sterol affinity was the basis for application of some saponins as cholesterol-lowering agents. Alfalfa saponins supplemented diet lowered total plasma

Table 3 Biological activity of saponins from different food plant species

Plant source	Activity
Allium	Cytotoxic, antifungal, enzyme inhibitory, cardioprotective, antispasmodic, anti-inflammatory, gastroprotective
Oat	Antioxidant, antifungal, increase intestinal permeability, inhibition of lactase activity
Beta vulgaris	Inhibition of glucose absorption, increase motor activity and blood serum α-lipoproteins, decrease blood serum pre-β-lipoproteins and erythrocyte osmotic resistance, inhibition of *Streptococcus mutans*, hemolytic
Chickpea	Antimicrobial
Soybean	Reduce growth performance and feed efficiency in fish, antioxidant activity, decrease of blood pressure, antiproliferative activity, modulate nutrient sensing pathways and metabolism, induce intestinal inflammation
Lentil	Inhibition of digestive enzymes
Tomato, potato	Antiprotozoal, anticancerogenic, antimalarial, liver cancer inhibition
Beans and peas	Inhibition of digestive enzymes, antitumor promoters in carcinogenesis, cytotoxic against cancer cells, regulation of cell proliferation, inhibitory effects against digestive enzymes, pancreatic lipase, and α-glycosidase
Alfalfa	Hemolytic, antifungal, antimicrobial, cholesterol reducing
Tea	Antimicrobial, active for gastrointestinal tumor, anti-inflammatory, anti-acne, preventing hemorrhoid, insecticidal, antialcoholic function, antioxidant
Garden balsam	Anti-hepatic fibrosis
Tiger's tail	Antidiabetic
Ginseng	Anti-inflammatory, anticancer, treatment of hypertension, vertigo and acute faucitis, cardiac remodeling induced by simulated microgravity, inhibiting platelet adhesion to injured endothelial cells, reduce all-cause mortality, myocardial infarction (MI), revascularization, rehospitalization for unstable angina, neuroprotective
Teas	Modulation of gastrointestinal system, anti-cancer, anti-inflammation, anti-microorganism, antioxidation, neuroprotection, hypolipidemic effects, foaming and detergence
Balloon lower	Enhances exercise function, skeletal muscle protein synthesis, and mitochondrial function
Quillaja saponaria	Immunomodulatory activity
Akebia	Reverses corticosterone hypersecretion in AD
Wild vegetable *Aralia elata*	Neuroprotective
Licorice	Anti-inflammatory
Amaranth vegetable	Hemolytic

cholesterol. The effect being prominent, when cholesterol was absent in the diet. But saponins supplementation did not exert any significant effect on the total liver lipids. Several possible mechanisms have been suggested to explain the hypocholesterolemic activity of saponins. However,

the exact mechanism of this activity remains unclear. The hypocholesterolemic effect of saponins could be directly correlated with their ability to decrease intestinal

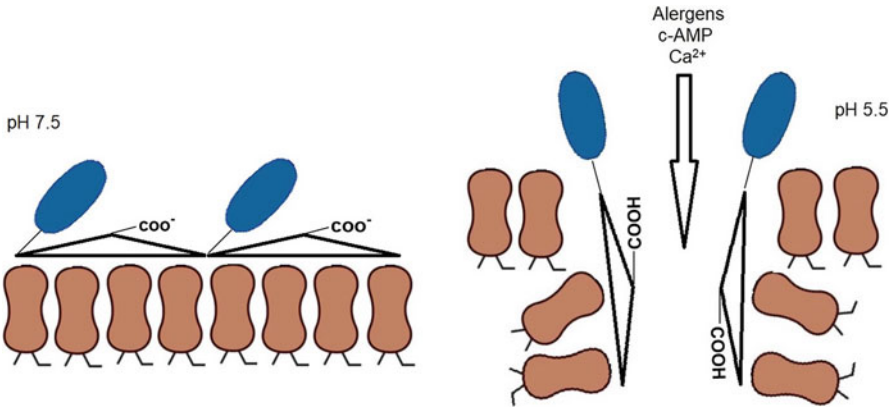


Fig. 15 Configuration of saponins at intestine membrane at pH 7.5 (left) and pH 5.5 (right). Triangles represent aglycone part, blue ellipse represent sugar chain. Brown parts represent hydrophobic intestine bilayer

cholesterol absorption and the increase the fecal excretion of bile acids. However, after ingestion, the nature and extent of the gastrointestinal microbial metabolism of saponins, i.e., the bioaccessibility and bioavailability of dietary saponin components are still lacking (Zhao 2016). Sterol affinity is also responsible for the moderation of the permeability of intestinal mucosa cells and change the absorption and excretion in the small intestine. Sterol binding and permeation of the epithelial barrier may have nutritional consequences. It may cause disorders in the absorption of important microelements or enhance the absorption of allergens (Fig. 15). The effect is strongly pH dependent. At neutral or slightly alkaline conditions, the aglycone (hydrophobic part) is bound to the epithelial cell layer, while in acidic condition, the aglycone penetrates the layer and disrupts the regular structure, making permeable holes in the intestine.

Similar mechanisms are most probably responsible for antimicrobial activity of saponins (Oleszek 2000). The general principle of the action of saponins on microorganism is their interaction with membrane sterols. Thus, the bacteria should not be sensitive to saponins, as their membranes are low in cholesterol. However, recent report on the saponin-sensitive and insensitive *Trichoderma viride* strains indicates that fatty acid composition of membranes may equally be important in this respect. The major effect of saponins on bacteria is the leakage of protein and certain enzymes from their cells.

Antifungal activity is generally demonstrated by saponins with triterpene and spirostanol skeleton. Furostanol saponins, which have bidesmosidic nature, do not complex with sterols and do not exhibit bacteriostatic or antifungal activity. Thus, the mechanism of antifungal activity seems to be dependent also on sterol affinity. Complexing with membrane sterols, proteins, and phospholipids seems to be a major mechanism that predisposes sensitivity of a microbe to saponins, but the effect is relatively nonspecific. Examples of antimicrobial, antifungal, antiviral, and cytotoxic activities of food plants, but also some medicinal plants, are presented in Tables 4, 5, 6, and 7.

Table 4 Antimicrobial activity of saponins from different plant sources. (modified after Oleszek 2000)

Plant source	Type of saponin	Microbe sp.	MIC
Aesculus hippocastanum	Triterpene	*Agrobacterium tumefaciens*	100 μM
		Rhizobium meliloti	100 μM
		Bradyrhizobium japonicum	100 μM
		Pseudomonas fluorescens	25 μM
Astragalus melanophrurius	Triterpene	Several species	Modest
Beta vulgaris	Triterpene	*Streptococcus mutans*	10 μg/ml
Capsicum annum	Steroidal	*Saccharomyces cerevisiae*	
		Bacillus cereus, B. subtilis	
Hedera helix	Triterpene	22 different bacteria	
Hedyotis nudicaulis	Triterpene	*Bacillus subtilis M45*	
Henricia laeviuscola	Steroidal	*Staphylococcus aureus*	
Hosta sieboldiana	Steroidal	*Debaryomyces polymorphus*	10 ppm
		Zygosaccharomyces rouxii	5 ppm
Medicago sativa	Triterpene	*Agrobacterium tumefaciens*	
		Corynebacterium insidiosum	
		Pseudomonas lachrymans	
		Corynebacterium michiganense	
		Xanthomonas campestris	
Medicago sativa	Triterpene	*Streptococcus aureus*	
		Pseudomonas aeruginosa	
Sanicula europaea	Triterpene	*Corynebacterium diphtheriae*	
Sapindus saponaria	Triterpene	*Pseudomonas aeruginosa*	*mm inhib*
		Bacillus subtilis	
		Cryptococcus neoformans	
Smilax aspera	Steroidal	*Cryptococcus neoformans*	
	Steroidal	8 bacterial strains	
Solenostemma argel	Unspec.	*Streptococcus* spp.	
		Escherichia coli	
		Bacillus anthracis	
		Staphylococcus aureus	
		Klebsiella pneumoniae	
Symphytum officinale	Triterpene	*Salmonella typhi*	100–200 μg/ 100 μl
		Staphylococcus aureus	
		Streptococcus faecalis	
		Escherichia coli	

Table 5 Antifungal activity of saponins from different plant sources. (modified after Oleszek 2000)

Plant source	Type of saponin	Fungus	MIC
Agave sisalana	Steroidal	*Aspergillus* spp.	
		Candida albicans	
		Rhodotorula glutinis	
		Saccharomyces cerevisiae	
Albizia lebbeck	Unspec.	*Macrophomina phaseolina*	32.8 μg/ml
Allium ampeloprasum	Steroidal	*Mortierella ramanniana*	10 μg/disc
Allium sativum	Steroidal	*Candida albicans*	100 μg/ml
Asparagus officinalis	Steroidal	*Trichophyton rubrum*	2–3 μg/ml
		Candida albicans	
Asparagus officinalis	Steroidal	*Candida, Cryptococcus* spp.	0.5–8 μg/ml
		Trichophyton, Microsporum spp.	
		Epidermophyton spp.	
Asparagus officinalis	Steroidal	Several fungi	
Avena sativa	Triterpene	*Fusarium avenaceum*	
Avena sativa	Triterpene	*Botrytis cinerea*	2.5 mg/ml
		Altenaria spp.	
		Fusarium lini	
Bellis perennis	Triterpene	*Candida* spp., *Trichophyton* spp.	
		Microsporum spp.	
		Aspergillus niger	
Camellia japonica	Triterpene	*Botrytis cinerea*	
		Pyricularia oryzae	
		Cochliobolus miyabeanus	
		Pestalotia longiseta	
Camellia japonica	Triterpene	*Trichophyton mentagrophytes*	25 μg/ml
		Epidermophyton floccosum	25 μg/ml
Camellia oleifera	Triterpene	*Trichophyton* spp.	0.125–1 μg/ml
		Epidermophyton floccosum	0.062–0.25 μg/ml
		Microsporum audouinii	10 μg/ml
Camelia spp.	Triterpene	*Pilicularia oryzae*	
		Cochliobolus miyabeanus	
Celmisia petriei	Triterpene	*Cladosporium cladosporioides*	
Cicer arietinum	Triterpene	*Penicillium digitatum*	
Chisocheton paniculatus	Steroidal	*Curvularia verruciformis*	
		Drechslera oryzae	
		Altenaria solani	
Clerodendrum wildii	Triterpene	*Cladosporium cucumerinum*	3.3 μg/plate

(continued)

Table 5 (continued)

Plant source	Type of saponin	Fungus	MIC
Dolichos kilimandscharicus	Triterpene	*Cladosporium cucumerinum*	2.5–5 μg/plate
Dracaena mannii	Steroidal	*Trichophyton* spp.	6.25–12.5 μg/ml
		Microsporum spp.	12.5–50 μg/ml
		Phialophora verrucosa	50 μg/ml
		Fonsecaea pedrosoi	25 μg/ml
		Cladosporium carrionii	12.5 μg/ml
		Exophiala jeanselmei	25 μg/ml
		Ramichloridium subulatum	25 μg/ml
		Candida albicans	25 μg/ml
		Candida tropicalis	100 μg/ml
		Trichosporon cutaneum	6.25 μg/ml
		Geotrichum candidum	12.5 μg/ml
		Rhodotorula spp.	25 μg/ml
Ecballium elaterium	Triterpene	*Botrytis cinerea*	
Hedera helix	Triterpene	*Candida albicans*	100 mg/kg
		Microsporum canis	0.5 mg/ml
Hedera helix	Triterpene	*Candida glabrata*	
Heteropappus altaicus	Triterpene	*Candida* spp.	
Heteropappus biennis	Triterpene	*Candida* spp.	
Lycopersicon esculentum	Steroidal	*Aspergillus*	1.0–0.0001M
		Candida albicans	
		Trichophyton	
Lycopersicon esculentum	Steroidal	*Gaeumannomyces graminis*	
Lycopersicon esculentum	Steroidal	*Septoria lycopersici*	
		Cladosporium fulvum	
Lycopersicon esculentum	Steroidal	*Corticium rolfsii*	100–150 μg/ml
Maesa ramentacea	Triterpene	10 fungal species	10–50 μg/ml
Malus meliana	Unspec.	Food preservation	
Medicago lupulina	Triterpene	*Trichoderma viride*	17–50 μg/ml
Medicago sativa	Triterpene	*Gaeumannomyces graminis*	3.5–7 μg/ml
Medicago sativa	Triterpene	*Sclerotium rolfsii*	
		Rhizoctonia solani	
		Aspergillus niger	
		Fusarium oxysporum	
		Cephalosporium gramineum	50 μg/ml

(continued)

Table 5 (continued)

Plant source	Type of saponin	Fungus	MIC
Medicago sativa	Triterpene	*Trichophyton mentagrophytes*	7–25 μg/ml
		Microsporum canis	5–10 μg/ml
		Epidermophyton floccosum	7–20 μg/ml
		Cryptococcus spp.	3.5–7 μg/ml
		Candida albicans	7 μg/ml
Medicago sativa	Triterpene	*Trichoderma viride*	17–50 μg/ml
Medicago sativa	Triterpene	*Trichoderma viride*	
		Candida albicans	
Medicago sativa	Triterpene	*Phytophthora cinnamomi*	
Medicago spp.	Triterpene	*Trichoderma viride*	
Mollugo pentaphylla	Triterpene	*Cladosporium cucumerinum*	1.5 μg/plate
Nicotiana tabacum	Steroidal	*Cladosporium cucumerinum*	
		Puccinia recondita	
Pisum sativum	Triterpene	*Fusarium solani*	150–300 μg/ml
Polemonium caeruleum	Triterpene	Yeasts, dermatophytes, molds	
Polygala vulgaris	Triterpene	*Ceratocystis ulmi*	
Primula acaulis	Triterpene	*Candida* spp.	
Rapanea melanophloeos	Triterpene	*Cladosporium cucumerinum*	1 μg/plate
Rudgea viburnioides	Triterpene	*Cladosporium cladosporioides*	
Ruscus aculeatus	Steroidal	*Aspergillus fumigatus*	
		Trichophyton mentagrophytes	
Sapindus mukurossi	*Steroidal*	*Cladosporium cucumerinum*	1.5 μg/plate
Serjania salzmanniana	Triterpene	*Cryptococcus neoformans*	8 μg/ml
		Candida albicans	16 μg/ml
Silphium perfoliatum	Triterpene	*Drechslera graminea*	10–1000 μg/ml
		Rhizopus nodosus	
		Rhizopus nigricans	
Solanum tuberosum	Steroidal	*Trichoderma viride*	
		Helmintosporium carbonum	
		Fusarium caeruleum	
		Cladosporium fulvum	
Solenostemma argel	Unspec.	*Asperillus* spp., *Penicillium*	
		Chrisoporium, *Candida* spp.	
		Cryptococcus neoformans	
		Mucor, *Rhodotorula*	
Solidago virgaurea	Triterpene	*Candida* spp.	

(continued)

Table 5 (continued)

Plant source	Type of saponin	Fungus	MIC
Trigonella foenum-graecum	Steroidal	*Rosellinia necatrix*	200 μg/ml
		Trichoderma viride	200 μg/ml
		Trichoderma harzianum	200 μg/ml
		Candida albicans	50–100 μg/ml
Trillium grandiflorum	Steroidal	*Candida albicans*	1.56–12.5 μg/ml
		Cryptococcus neoformans	
		Saccharomyces cerevisiae	
		Aspergillus spp.	
		Trichophyton mentagrophytes	
Zygophyllum spp.	Triterpene	*Verticillium albo-atrum*	
		Fusarium oxysporum	
Yucca schidigera	Steroidal	*Saccharomyces* spp.	31–62 μg/ml
		Candida famata	31.3 μg/ml
		Hansenula spp.	31.3 μg/ml
		Cryptococcus spp.	31.3 μg/ml
		Pichia spp.	31.3 μg/ml
		Debaromyces spp.	31–125 μg/ml
		Zygosaccharomyces spp.	31.3 μg/ml
		Candida albicans	62.5 μg/ml
		Trichophyton spp.	15.6–31.3 μg/ml
		Sabouraudites canis	31.3 μg/ml
		Epidermophyton floccosum	31.3 μg/ml

It has been proven that saponins exert positive influence on ruminants. *Y. schidigera* plant extract has been found to improve growth, feed efficiency, and health in ruminants. Similarly, *Quillaja* and lucerne saponins have been investigated as agents increasing the efficiency of in vitro rumen-microbial protein synthesis, significantly reducing the total protozoa count in the rumen and decreasing feed protein degradability. The influence of saponins on protozoa resulted from their binding with sterols present on the protozoa surface, which lead to the destruction of the cell (Szumacher-Strabel and Cieślak 2010).

Beneficial effect of saponins on ruminants is associated also with their ability to bind ruminal NH_4 at its high concentration, and release NH_4 again, when it is at low concentration. It provides a continuous, adequate supply of NH_4 for microbial protein synthesis (Francis et al. 2002).

Saponins in animal feed are very helpful in mitigation of methane emission from animal excretes. This emission is very harmful for environment, because methane is one of the main greenhouse gases (GHG). Moreover, loss of methane from the fermentation processes occurring in the rumen, deteriorates feed utilization, and thus

Table 6 Antiviral activity of saponins. (modified after Oleszek 2000)

Saponin	Plant source	Virus type
Chikusetsusaponin III	*Panax japonicum*	HSV-1
Deltoside	*Allium nutans*	TMV
Extract	*Verbascum thapsiforme*	HSV-1
Glycyrrhizin	*Glycyrrhiza glabra*	HAV
		VZV
		HIV-1
		HIV-1, HSV-1
		HIV
		HIV-1
Holoturinosides	*Holothuria forskalii*	VSV
Oleanolic acid glycosides	*Calendula arvensis*	VSV, HRV
		HRV-1B, VSV
Protoprimulagenin glycosides	*Anagallis arvensis*	HSV-1, AV-6, VSV
Quinovic acid glycosides	*Uncaria tomentosa*	HRV-1B, VSV
Saikosaponin a	*Bupleurum falcatum*	HSV
Soyasaponin (Bb)	*Glycine max*	HIV-1
Soyasaponin I and II	*Glycine max*	HSV-1
Soyasaponin B1 and B2	*Glycine max*	HIV
Spirostane and furostane glyc.	*Tamus communis*	HRV-1B, VSV
	Asparagus cochinchinensis	
Taurosid I		HIV-1
Tormentic acid, euscafic acid		HSV-1
Oleanolic acid glycosides		Poliovirus
Triterpene saponins	*Chenopodium anthelminticum*	Influenza A2
	Callistephus chinensis	
	Glycyrrhiza glabra	
Zingibroside R1	*Panax zingiberensis*	HIV-1

decreases the economic effect of animal maintenance (Szumacher-Strabel and Cieślak 2010). Many studies proved that supplementation of feed with saponins reduced the methane production in the rumen, due to the negative effect on protozoa population, providing hydrogen for methanogens. Mitigation of methanogenesis results from a decreased methanogenic activity, without changing the total methanogen numbers, because methanogens cells do not include cholesterol (Cieślak et al. 2013).

Dietary saponins, particularly from lucerne, were often suspected of causing ruminant bloat, though clear experimental proof for this is lacking in the literature. There were some literature data showing bloating in animals fed with alfalfa, but there is not hard proof that saponins are responsible for that. However, in experiment with hamsters, where alfalfa saponin solutions were orally introduced, the production of gases in the intestine was observed, causing discomfort for experimental animals.

Table 7 Cytotoxic activity of saponins. (modified after Oleszek 2000)

Saponin	Plant species	Cytotoxicity	Concentration
Arginine C	*Palaquium formosanum*	prostate	13.8 mmol
Diosgenin, dihydrodiosgenin	*Dracena afromontana*	KB cells	10 μg/ml
Diosgenin glycosides	*Paris polyphylla*	KB cells	0.16–0.29 μg/ml
		P-388	0.22–0.44 μg/ml
		L-1210	0.14–043 μg/ml
Diosgenin glycosides	*Balanites aegyptiaca*	P-388	0.21–2.4 μg/ml
Echinocystic acid octaglyc.	*Entada phaseoloides*	L-5178 Y	0.83 μg/ml
Ginsenoside R_{b2}	*Panax ginseng*	RLE, B16	Not effective
		HRA	10–100 μmol
α-Hederin	*Hedera helix*	B 16	5 mg/ml
Holestane glycosides	*Holothuria forskalii*	P-388	0.38–0.46 μg/ml
Oleanolic acid diglycoside	*Panax zingiberensis*	MT-4	46.2 μmol
Pectiniosides A-F	*Asterina pectinifera*	L 1210	8.8–11 μg/ml
		KB	10–11.5 μg/ml
Saikosaponins	*Buphlerum wenchuanese*	PLC/PRF/5	20–50 μg/ml
"Sho-saiko-to" medicine		Hep-G2	
Saikosaponi-acetylglycosyl		P-388	<5 μg/ml
Sarasinoside A1	*Asteropus sarasinosum*	P-388	2.8 μg/ml
Sarasinosides D-G		A-549	7.4 μg/ml
		HT-29	4.55 μg/ml
		P-388	3.62 μg/ml
		B 16-F 10	9.62 μg/ml
Solamargine	*Solanum* spp.	PLC/PRF/5	1.53 μg/ml
Khasianine		PLC/PRF/5	8.60 μg/ml
Spirostanol glycosides	*Chamaedorea linearis*	L 1210	32 μmol
Trillin	*Dracaena afromontana*	KB cells	100 μg/ml
Triterpene glycosides	*Dysoxylum cumingianum*	MOLT-4	0.0045–0.0062 μg/ml
Triterpene glycosides	*Myrsine australis*	P-388	0.85 μg/ml
Tubeimoside 1	*Bobolstemma paniculatum*	GOTO	0.24 μmol/l
		A-172	0.15 μmol/l
		PANC-1	0.27 μmol/l
		COLO 320 DM	0.43 μmol/l
Yamogenin glycosides	*Balanites aegyptiaca*	P-388	0.21–2.40 μg/ml
Zingiberoside R1	*Panax zingiberensis*	MT-4	84.4 μmol

Some saponins may influence on activity of different enzymes involved in digestion or other metabolic processes. Total saponins of radix and flower of ginseng, which have high content of saponins, were able to inhibit the

inflammatory-related transcriptional activities and the related mRNA expression of IFNα, TNFα, il-6 and TGFβ, as well as induce anti-oxygen NrF2 activities.

The research on commonly consumed food legumes produced in China proved their inhibitory effects against digestive enzymes, pancreatic lipase, and α-glycosidase. Saponin extracts from the adzuki bean and rock bean exhibited inhibitory effects against α-lycosidase. The results indicate that adzuki bean is one of the best target beans for further study on their anti-obesity and antidiabetic effects.

The avenacosides present in *Avena sativa* seeds inhibited the lactase activity significantly in vitro. However, in vivo studies showed no or small effects due to the low avenacosides concentration found in oats. The in vitro hydrolysis of starch by α-amylase was increased in the presence of saponins, probably due to their detergent effect. Thus, the in vitro studies showed that the avenacosides could influence the enzyme activities.

Saponins may show some features discouraging feed intake by animal. Some of them are recognized as bitter compounds, while the others, e.g., zanhic acid glycosides from alfalfa show strong throat-irritating activity. However, since the concentration of saponins in most food and feedstuffs is low, these effects are not of great importance in their acceptance. Inclusion of purified alfalfa saponin in Bengalgram diet, showed no interference with food intake and food efficiency ratio during 47 days of feeding experiment. Saponin supplemented diet lowered total plasma cholesterol. This effect was prominent when cholesterol was absent in the diet. The hypocholesterolemic effect of saponin could be directly correlated with their ability to decrease intestinal cholesterol absorption and the increase of the fecal excretion of bile acids.

Most of research performed on the influence of saponins on animal performance can be question. The reason for that is poor characterization of feedstuff. Instead of pure saponins, rather poorly characterized plant material is being used in experiments and, in fact, it is difficult to precisely say which plant component is responsible for the observed effect.

Many research data show that saponins generally are not absorbed from the digestive tract into blood stream. In the chamber experiment with segments of proximal rats' small intestine, it was found that avenacosides from oats do not pass across intestinal epithelium. No saponins were, however, detected in feces collected from rats given avenacosides. In experiments with chicken, rats, and mice fed soybean saponins, no saponins or sapogenins were detected in the blood indicating they were not absorbed. At the same time, saponins in all three animal species were detected in small intestine, but in the cecum and colon, only sapogenins were found. This indicated that saponins were hydrolyzed by colonic microflora or by enzymes in the lower part of intestine.

In experiments with zebrafish fed soybean saponins, some metabolic disturbances and growth reduction were observed. Saponins fed at the concentration of 5 and 10 g/kg of diet significantly reduced growth performance and feed efficiency, and damaged the morphology of intestinal mucosa. Saponins also increased the expressions of key metabolic enzymes involved in glutamine synthetase, glutamate

dehydrogenase and lipolysis, hormone-sensitive lipase, and lipoprotein lipase. This documented that saponins modulate nutrient sensing pathways and metabolism.

In experiment with hypertensive rats, feeding with soybean saponins 80 mg/kg of body weight per day for 8 weeks significantly decreased blood pressure. The mechanism of this activity remains unclear.

However, more epidemiological and clinical studies are required for the proper validation of these activities. For better understanding of these effects, more precise saponin products should be used and more sophisticated methods of evaluation of intact saponins, as well as their metabolites and degradation products, are required.

32.4 Benefits for Humans

As discussed above, saponins may exert differential biological activities depending on their structures. It is also quite evident that, when consumed in human diet, they show no toxicity at the doses present in foodstuffs. This is mostly due to the fact that their concertation in foodstuffs is rather low. Besides, their high molecular weight, poor stability under stomach condition and poor membrane permeability make them low bioavailable. It was suggested by recent research that the bioavailability can be enhanced by the application of different "absorption enhancers," which act as opener of cellular pathways of absorption. Bio-adhesive preparative containing saponins extend the time of preparation effects on target sites, increase the contact with the absorption membrane, change membrane fluidity, and increase saponin penetration to the intestinal epithelial cell, promoting absorption and oral availability (Li et al. 2017). These enhancers can be natural components of some foodstuffs such as fatty acids, phospholipids, as well as calcium chelators, surfactants, bile salts, and chitosan derivatives. Some other means of enhancing saponin absorption were also suggested. That includes usage of liposomes and nanoparticles as physical vehicles or esterification of saponins with fatty acids to increase their hydrophobicity. This may suggest that evaluation of the absorption level from the food matrix can be different from those observed with pure saponins. It should be also mentioned that bioavailability of saponins can be different for individuals. The paracellular permeability of intestinal epithelium can be individual characteristic or can be modified by some health disorders, e.g., diabetes. The bioavailability of saponins in different animals is also different. Some of the observed values were as low as 0.1% for saponins from ginseng (ginsenosides) in rats, or 3% in dogs, and as high as 90% for the sapogenin from licorice (glycyrrhetic acid), also in rats.

The bioavailabity of saponins may also be changed by the processing of saponin-bearing foodstuffs. It was documented that bioavailability of soybean saponins present in bread was in the range of 30–91%, depending on the structure.

The seeds of legumes, which are rich in saponins, are usually presoaked and cooked, which is accompanied by leaching of saponins. The level of leaching is dependent on the time of the process and on the seed to water ratio. Pre-soaking enhanced subsequent thermally induced degradation of the saponins and caused a high loss of the contents during the cooking processes. It was shown that in the

cooked lentils, bioaccessibility of soyasaponins was in the range of 9–10%. Another research showed that accessibility of saponin from lentil, soybean, fenugreek, and quinoa ranged between 30% and 100%. Several factors can influence this accessibility including concertation of saponins in the extract, presence of other food components, type of saponin, as well as the ratio of saponin to sapogenin in the matrix (Hierro et al. 2018).

The stability of saponins in vegetarian, broccoli-based bars incorporating chickpea, soy, and faba beans as protein sources after being subjected to different cooking methods were studied. Commonly domestic ways of bars preparation were microwaving, frying, frying and microwaving, steaming, and baking. It was shown that soaking and peeling of checkpeas and faba bean reduced saponin content by 8% and 35%, respectively. Particularly, DDMP saponins were affected (Barakat et al. 2015).

The major benefit of saponins in human nutrition seems to be their cardioprotective activity. Thus, it seems reasonable to introduce saponin-bearing plants such as alfalfa, onion, garlic, soybean, beans, and ginseng to inhibit the intestinal absorption of cholesterol and reduce serum cholesterol level. High saponin extracts from these plant species, as well as from some medicinal plants (*Terminalia arjuna*, *Clematis species*, *Glycyrrhiza glabra*, *Ilex cornuta*, *Crataegus oxyacantha*, *Astragalus membranaceus*), enriched with "absorption enhancers," may be a good solution for food supplements in routine intake for cardiac protection. Semisynthetic "designer saponins" with high saponin content have been of interest, and several patents were received.

Another possible feature benefit for consumer is vasorelaxant activity of saponins. The ginsenosides Rg1, Re, Rb, Rc, and Rg3 showed vasorelaxant activity by inhibiting Ca^{2+} influx via receptor-operated Ca^{2+} channels in vascular smooth muscle cells. Out of these, the ginsenoside Rg3 emerges as the most potent vasodilatory agent to open the Ca^{2+} activated K^{+} channel. Other activities of saponins in the prevention of human diseases are presented in Table 3.

32.5 Safety: Toxicity and Side Effects

Despite many beneficial effects of saponins on the human and animal health, the problem of their toxicity is still considered and important. As with all others substances, also in the case of saponins, the toxicity depends on the dose and the route of entry of chemicals into organism. Moreover, it should be mentioned that saponins, as the large group of compounds, varied much in the toxicity (George 1965).

It is commonly known that saponins are poison for fish. Nonetheless, the results of many studies proved that they are practically nontoxic to man upon oral ingestion, because they normally remain within the digestive tract (Oakenfull 1981). The problem with safety of saponins can occur, for example, in the case of severe poisoning of gastrointestinal lesions or irritation of the gastrointestinal tract by some causes, allowing saponins to enter into the blood stream. At that time, the intravenous lethal dose can be one thousandth of the lethal oral dose (George 1965).

The result of penetration of saponins into the blood may be liver damage, hemolysis of red blood cells, respiratory failure, convulsions, and coma. Therefore, it is recommended that people using drastic purgatives or having inflamed intestines or cirrhotic livers should avoid all foods containing saponins. Moreover, saponins can moderate the permeability of intestinal mucosa cells and change the absorption and excretion in the small intestine. It may cause disorders in the absorption of important microelements or enhance the absorption of allergens (Lásztity et al. 1998). Simultaneously, it is claimed that the saponins from soybeans, lucerne, or *Quillaia* are safe for short-term human-feeding trials at levels of below 50 mg kg^{-1} body weight per day (Oakenfull 1981; Lásztity et al. 1998).

Many saponins exhibit cytotoxic activity, which is a promising property for their anticancer application. Unfortunately, the hemolysis of red blood cells is a major drawback for the clinical development of saponins as antitumor agents (Gauthier et al. 2009). Structure–activity relationship (SAR) studies have recently been undertaken in order to explain the differences in the action between particular saponins and to select the most favorable compounds devoided of undesirable toxicity. It has been proven that both the nature of the aglycone and the osidic part are important for biological activity of saponins. Moreover, bidesmosidic saponins, bearing sugar moieties at both C-3 and C-28 positions, were generally less hemolytic toward erythrocytes than C-3 monodesmosides. Furthermore, the presence of nonnatural linkages (1,2-cis-glycosides) have detrimental effects both for the hemolytic and cytotoxic activities of saponins. However, there is no evident correlation between the hemolysis of red blood cells and cytotoxicity of saponins (Gauthier et al. 2009).

32.6 Marketed Products

The beneficial physicochemical and biological properties of saponins have been successfully exploited in a numerous commercial applications in food (Güçlü-Üstündağ and Mazza 2007). In some countries there are regulations describing the level and the source of saponins allowed to be used in food products. The two major commercial sources of saponins are *Quillaja saponaria* and *Yucca schidigera* extracts. As early as 1962, Quillaya saponins have been approved to be used as emulsifiers and foaming agents at maximal concentrations of 20 ppm in the UK. *Quillaja* extract is classified also by the European Union as a foaming agent for use in water-based, flavored nonalcoholic drinks. Its symbol on the list of ingredients is E999. Similarly, *Quillaja* and *Yucca* extracts rich in saponins received Generally Recognized as Safe (GRAS label) issued by Food and Drug Administration in the USA. *Quillaja*, *Yucca*, soybean, and enju saponins are accepted in food products also in Japan.

As natural nonionic surfactants, saponins find widespread use in food as emulsifiers (Oleszek and Hamed 2010). The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and water-soluble side chain. Hydrophilic part of saponins is built from a sugar chain, which can differ in the length, branching, substitution, and composition (glucose, galactose, rhamnose, arabinose, xylose,

apiose, and uronic acid), while the lipophiles may have a steroidal or triterpene structure. This is why saponins are recognized as nonionic surfactants. The strongest surfactant activity is showed by saponin with one sugar chain. An increase in the number and length of sugar chains decreases foaming characteristics, or sometimes, no foaming effect is observed. A great advantage of saponins, as emulsifiers over other synthetic emulsifiers, is their salt-free nature, which makes them less likely to be affected by alkaline or acid conditions (Zhu et al. 2019). Thus, saponins from different sources are being used in following food processing technologies:

- As emulsifiers of oil-based flavors for candy
- As substances preventing precipitation in a protein containing liquid composition
- As substances helping to prevent oil separation in mayonnaise
- As a leavening agent in the bakery industry
- As compounds increasing stability of cream when added to coffee
- As a natural dispersing agent for waxes used in food coatings

Application of *Yucca* extract to sugar-candy foam at the saponin concentration equivalent to 50 and 100 ppm proved its better foaming parameters that eggs whites. This extract had a beneficial effect on dry matter content, density, and porosity of sugar-candy foam products. Moreover, since *Yucca* extract contains also strong antioxidants, the shelf life of sugar-candy foams were substantially prolonged (Sucharzewska et al. 2003).

The main food products providing saponins to human and animal diet are vegetables containing these compounds such as beans and legumes, garlic, asparagus, spinach, oats, quinoa, and amaranth and lucerne sprouts. Despite the fresh vegetables available in the market, there are also products of their processing. The most popular prepared products containing saponins are tofu, falafels, and tempe. Saponins are present also in protein concentrates prepared from saponin-containing plants, mainly soya protein isolates, and leaf protein from lucerne.

According to the newest reports of Patra et al. (2018), the species *Panax ginseng* is the most widely cultivated and marketed species among phytochemicals-rich plants all over the world. This plant is distributed across approx. 35 countries, where market is subdivided based on its application in food products and dietary supplements, but also in pharmaceuticals, personal and oral care products. The worldwide ginseng production is estimated at more than 80,000 t. In 2015, the ginseng's global market of dietary supplements was valued at over US$ 123 billion. Moreover, it is assumed that the market's demand for ginseng will continue to remain strong throughout the 2016–2026 forecast period (Patra et al. 2018). Nowadays, there is the highest demand for the powdered form of ginseng, due to its extensive consumption as an energy booster.

Some saponins from non-food plants find application as food supplements. A good example is *Tribulus terrestris*, which is used in the folk medicine against sexual impotence, edemas, abdominal distention, and cardiovascular diseases (Kostova et al. 2002). Many pharmaceutical preparations and food supplements based on the saponin fraction from this plant are on sale worldwide. Another widely

Table 8 Plant sources for industrially utilized saponins (Böttcher and Drusch 2017)

Plant source	Common name	Use
Agave sisalana	Sisal, Henequen	Hecogenin source
Balanites aegyptiaca		Diosgenin source
Chlorogalum pomeridianum	Soaproot, California soap plant	Amolonin source surfactant
Costus speciosus		Diosgegnin source
Digitalis lanata	Purple Foxglove	Digitonin source
Digitalis purpurea	Digitalis	
Dioscorea composita	Yams, Barbasco	Diosgenin source
Dioscorea terpinapensis		
Dioscorea spp.		
Glycine max	Soybean	Soyasaponin source
Quillaya saponaria	Quillaya	Soaps, foaming agents
Chenopodium quinoa	Quinoa	Brewing, cosmetics, and detergent production
Smillax spp.		Smilagenin and sarsapogenin source
Solanum spp.		Solasodine source
Trigonella faenum-graecum	Fenugreek	Diosgenin source
Yucca schidigera	Mohave Yucca	Soaps, foaming agents
Avena sativa	Oats	Emulsifier

known food supplement for sexual potency and for body builders is produced from Bucher's broom (*Ruscus aculeatus)*, a plant containing ruscogenin and some other saponins.

Number of saponin containing plant species is being widely used for the production of saponin raw materials for pharmacy, cosmetic, and food industries. These are presented in Table 8.

32.7 Patents

The presence of saponins in food, as natural components and as additives, brings benefits both for consumers and for producers of food. The growing interest in their beneficial properties is manifested by the issuance of more and more patents in this sector. Two hundred eighteen patents, published in the last 5 years, have been recorded. The most patented plant was *Panax ginseng* and other species from this genus. Moreover, many patents concerned also *Camelia* sp. (Fig. 16). As regards the country, the greatest activity in patenting was recorded in China, South Korea, the USA, Russian Federation, and Japan (Table 9) (ISI Web of Knowledge, Derwent Innovations Index Database 2018).

Many patents on saponins concern manners of their extraction and isolation in the context of subsequent usage in food, as well as the methods of their determination

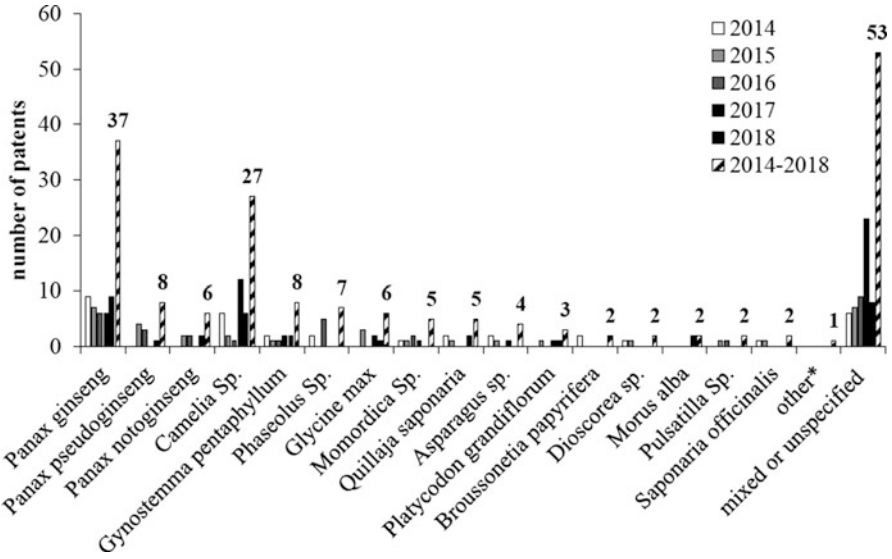


Fig. 16 The number of patents depending on the plant species in 2014–2018 based on ISI Web of Knowledge, Derwent Innovations Index Database (2018). Other plant, for which only one patent during the period of 2014–2018 was recorded: *Alopecurus myosuroides*, *Akebia* sp., *Beta vulgaris*, *Bupleurum* sp., *Cannabis sativa*, *Cicer arietinum*, *Cistanche salsa*, *Cucurbita* sp., *Derris eriocarpa*, *Epimedium* sp., *Ganoderma* sp., *Gossypium* sp., *Hedychium flavescens*, *Hosta* sp., *Ipomoea batatas*, *Kosteletzkya virginica*, *Lilium* sp., *Monascus* sp., *Myriopteron extensum*, *Pandanus* sp., *Polygonatum odoratum*, *Polygonatum officinalis*, *Sarcodon imbricatus*, *Siraitia grosvenorii*, *Solanum lycopersicum*, *Spartina alterniflora*, *Tupistra chinensis*, *Vaccaria pyramidata*, *Vitis* sp., *Yucca* sp., *Ziziphus jujube*

Table 9 Countries of inventors of saponins' application in food (2014–2018) (ISI Web of Knowledge, Derwent Innovations Index Database 2018)

Country	Number of patents	Share in total (%)
China	154	70.6
South Korea	45	20.6
United States of America	7	3.2
Russian Federation	7	3.2
Japan	5	2.3

and quantification in food and feed. Nonetheless, the vast majority patents published in recent years presented the way of application of saponins as food ingredients and additives. The most frequently patented was the usage of saponins as emulsifiers in food and beverages. Moreover, a lot of inventions related to saponins in food, were based on their beneficial effect on humans and animals health.

Panax ginseng saponin are useful in beverages and emulsified food but also in the production of healthcare and functional food. Such food containing ginseng saponins is recommended for preventing and treating cancer, cardiovascular and liver

diseases, as well as overcoming physical fatigue. Moreover, ginsenosides are patented as ingredients of food for improving and restoring skin appearance and elasticity, preventing skin aging, providing beautifying effect, as well as treating depression and anxiety.

Saponins from *Panax notoginseng* were used for preparing plant salt useful for promoting digestion, and as the ingredients of functional food for treating Parkinson's disease, reducing blood pressure and lowering blood fat.

The patents of tea saponins concern their use as emulsifiers in production of beverages and food but also in preparation of fertilizers and organic insecticides. A huge number of patents concern the application of tea saponins as components of animal feed. Feed enriched in tea saponins increases animal immunity, preventing diseases and promoting appetite.

Next, the most frequently patented plant being the source of saponins for composition of livestock feed is *Gynostemma pentaphyllum*. The *Gynostemma* saponins fed to chicken improve their immunity. Other patented applications of this plant concerned the production of healthcare food used for treating the obesity, diabetes type II, and hyperlipidemia. *Gynostemma* saponins are useful also for preparing composition for improving intestinal flora and promoting characteristic microbial colonization.

Popular food plant rich in saponins is also soybean. According to one of the latest patents, food and feed enriched in soybean saponins can prevent and treat osteoporosis, due to the improvement of formation and differentiation of osteoblasts. Moreover, soybean extract containing saponins was patented as an agent enhancing taste of food, e.g., sauce and soup. There are few patents, concerning food composition with saponins of other plants belonging to *Fabaceae* family, such as adzuki bean, mung bean, kidney bean, etc. Such food exhibits anticancer and antioxidant activity, prevents osteoporosis, enhances immunity, reduces blood cholesterol, induces appetite, and improves functioning of pancreas.

Saponins are important components of various compositions of food and drinks for treating hyperglycemia and other diseases associated with the sugar metabolism. Tomato saponins were used for preparation of oral liquid or healthcare food for treating of insulin resistance and sugar tolerance, reducing blood sugar, and increasing insulin sensibility. Moreover, there are patents providing the usage of *Derris eriocarpa* and samara saponins in sweetening composition for food or drink.

Among other, the most interesting patented applications of saponins, the usage of *Yucca* saponins as components of sausage and ham, should be mentioned. The inventors claimed that these products have unique taste and flavor, antimicrobial activity, antioxidant activity, inhibit growth of spoilage bacteria. Moreover, saponin component is easily consumed by consumers and promotes health of people, as well as is effective for prevention and treatment of multiple cancers.

One of the worth mentioning patents is also application of lily saponins for production of sleep-aiding honey bread. Thanks to synergic effect of lily saponins, the fructus gardeniae oil and the liensinine, the honey bread is fresh and sweet, delicious, and convenient to eat and has effects of shortening fall-asleep time, prolonging sleep time, and resisting convulsion. Moreover, the bread can effectively

improve the sleep quality of an insomnia patient; it is eaten for a long time. Similar properties were exhibited by saponins of *Ziziphus jujube* (spina date). Extract from its seeds was the component of patented healthcare food composition, useful for improving sleep.

Many inventors did not precise the origin of saponins, or used the mix of saponins from various species. It makes up one fourth of patents from the years of 2014–2018. Apart from functional healthy food, mixed or unspecified saponins were applied mainly for preparing high strength food bag, membranes useful, e.g., for coating on fried foods, degradable films for packaging of food products, or coating composition useful for preserving foodstuffs. Furthermore, mixture of various saponins was applied also in the composition of natural food preservatives.

32.8 Perspectives

Saponins due to their wide range of biological activities are interesting compounds with high potential to be applied in food supplement, medicinal food, and food processing industries. The last function seems to be connected to emulsifying abilities of most of these compounds. As they are generally not toxic to humans, their application as natural emulsifiers should be explored. Many researches have been conducted during last decades on these aspects.

In regard to their health-promoting activities, it has been very important to established best practices of food preparation to increase their accessibility. As the food matrix of individual foodstuffs containing saponins is different, each plant species should be treated separately or in the mixture with other accompanying food components.

Important issue in saponin health-promoting activity is their bioavailability, which is a consequence of many processes the saponin molecules are undergoing when enter digestive tract. It is known that hydrolytic enzymes present in gut or microbiota enzymes hydrolyse glycosidic forms to hydrophobic aglycones. Nonetheless, it still remains not clear which form is better absorbed and active. The cloned BlBG3, a GH3 β-glucosidase from *Bifidobacterium longum*, was proved to be microbiota enzyme processing ginseng saponin. However, structurally divergent saponins may undergo different mechanisms of these conversions and should be research in detail separately. Further studies on understanding factors modulating gastric transformations of saponins seem necessary.

Interesting option for increasing saponins bioavailability is the application of different kinds of absorption enhancers. Development of easy and practical enhancers is of high priority.

32.9 Cross-References

► Alkaloids in Diet
► Dietary Triterpenoids

► Oligosaccharides in Food
► Soy Isoflavones

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Dietary Phytoecdysteroids

33

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Abstract

Phytoecdysteroids are polyhydroxylated steroids which are widely distributed in the plant world and are present in significant amounts in 5–6% of plant species. Their major role in the plant is probably to deter invertebrate predators, but ecdysteroids also have many beneficial effects in mammals and are attracting attention as therapeutic and nutraceutical agents. Four hundred analogues have been identified so far from plant sources, but 20-hydroxyecdysone is the most frequently encountered and is often the major analogue present. Here we consider the occurrence of phytoecdysteroids in food plants and the human diet and how this might change in the future against the backdrop of what we currently know about biosynthesis of these compounds in plants and their bioavailability, metabolism, and biological activities in mammals. Finally, we discuss the medical and pharmaceutical potential of these molecules, particularly in the area of muscle wasting diseases and diabetes, and indicate which areas of fundamental research require focused study.

Keywords

20-Hydroxyecdysone · Anabolic · Antidiabetic · Bioavailability · Biosynthesis · Dietary intake · Metabolism · Quinoa · Spinach · Steroid · Structure-activity relationship

Abbreviations

20E	20-hydroxyecdysone
2d20E	2-deoxy-20-hydroxyecdysone
4E-BP1	4E-binding protein 1
ADMET	adsorption, distribution, metabolism, excretion, toxicology
AjuC	ajugasterone C
CNS	central nervous system
Cyast	cyasterone
DHT	dihydrotestosterone
DMD	Duchenne muscular dystrophy
E	ecdysone
E2	estradiol
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IGF-1	insulin-like growth factor 1
Ino	inokosterone
IntA	integristerone A
LC	liquid chromatography
LD_{50}	dose bringing about 50% mortality
MakA	makisterone A
MS	mass spectrometry
mTORC1	mammalian (or mechanistic) target of rapamycin complex 1

NMR	nuclear magnetic resonance spectroscopy
PI3K	phosphoinositide kinase-3
PoA	ponasterone A
PolB	polypodine B
Pter	pterosterone
RIA	radioimmunoassay
Rub	rubrosterone
SAR	structure-activity relationship
TLC	thin-layer chromatography
Turk	turkesterone
UV	ultraviolet

33.1 Introduction

There are believed to be 50,000 edible plant species among the estimated 300,000 terrestrial plant species, but only a few hundred contribute significantly to the human diet. Further, fewer than 20 account for 90% of the plant food intake of humans, with just 3, maize, rice, and wheat, accounting for >50% of the calorific intake (https://www.worldatlas.com/articles/most-important-staple-foods). While this perhaps makes the plant secondary compounds of those staple foods particularly relevant, it should not be allowed to obscure the contribution that the secondary compounds present in the minor food species might contribute to the diet, since their phytochemical contents and geographical preponderance vary enormously, and relatively small intakes of particular secondary compounds can have a marked impact on the physiological status of the human body.

Ecdysteroids are a family of steroid hormones, which were initially identified in insects and crustacea as hormones regulating molting, development, and reproduction (Koolman 1989). These zooecdysteroids have since been found to be present in other arthropods and invertebrates, but their roles are not so clearly elucidated. Shortly after the characterization of the structures of ecdysone (previously known as α-ecdysone) and 20-hydroxyecdysone (formerly known as β-ecdysone, ecdysterone, or crustecdysone) in the mid-1960s (Fig. 1), four research groups almost simultaneously discovered that ecdysteroids were also present in certain plant species (summarized in Nakanishi 2006), where they are referred to as phytoecdysteroids, in far higher concentrations than those found in invertebrates. To date, ca. 500 ecdysteroid analogues have been identified (Ecdybase; Lafont et al. 2002) from invertebrates, plants, and fungi (mycoecdysteroids), although the vast majority of these have been isolated from plants, reflecting the much higher concentrations found in ecdysteroid-containing plants (typically 0.1–1% of the dry weight but reaching 3.2% of the dry weight in the case of bark of *Diploclisia glaucescens*; Bandara et al. 1989). 20-Hydroxyecdysone is the most frequently encountered and major ecdysteroid analogue in arthropods and plants. Accumulating evidence supports the role of phytoecdysteroids in the reduction of invertebrate predation on the plants containing them either by endocrine disruption on ingestion

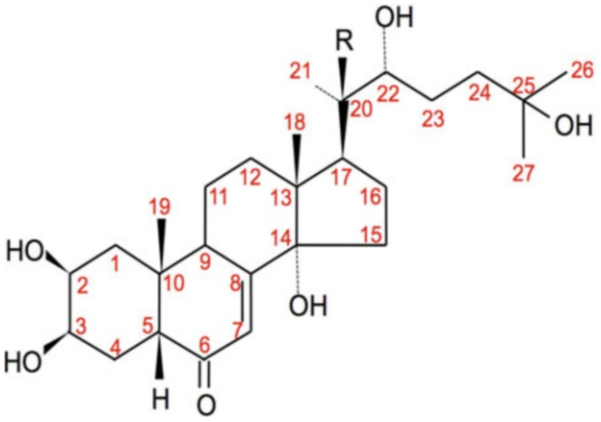

Fig. 1 Structures of ecdysone (R = H) and 20-hydroxyecdysone (R = OH), showing the stereochemistry and the standard numbering system

of the plant material or by deterrence following contact or ingestion of a small sample of the plant. This has led to the suggestion that phytoecdysteroids might be used to enhance the protection of crop species against predation. Indeed, it appears that most, if not all, plant species retain the genetic capacity to produce ecdysteroids, but that differential regulation of the as yet not fully elucidated biosynthetic pathway determines whether a particular species will accumulate ecdysteroids, at what levels within different parts of the plant and what the mix and proportions of various ecdysteroid analogues (the ecdysteroid profile) will be (Dinan et al. 2001).

The attractiveness of this idea is considerably enhanced by the lack of toxicity of ecdysteroids to mammals and by a plethora of essentially beneficial pharmacological and physiological effects in mammals which have been ascribed to phytoecdysteroids on ingestion or injection (reviewed Dinan and Lafont 2006). Mammals do not have the capacity to synthesise ecdysteroids de novo, nor do they possess homologues of the arthropod ecdysteroid receptor proteins (EcR and USP/RXR). Also, ecdysteroids, which differ significantly from vertebrate steroid hormones in polarity, bulk and shape, do not appear to interact with the vertebrate steroid hormone nuclear receptors (Báthori et al. 2008). However, exogenous ecdysteroids, like other dietary xenobiotics, are absorbed, distributed, metabolized, and excreted by mammals. In vivo and in vitro studies indicate that ecdysteroids modulate mammalian cell activities rapidly (in seconds to minutes) by interaction with membrane receptors and/or ion channels, which are in distinct contrast to the direct genomic regulation via nuclear receptors in arthropod cells (over hours to days). In view of the diversity of potential pharmacological benefits of ecdysteroids in mammals, there is a rapidly growing interest in assessing these compounds for use in a variety of medical conditions, especially those which involve muscle wasting (sarcopenia, cachexia, Duchenne muscular dystrophy, etc.).

In this review we shall consider the occurrence and identity of ecdysteroids in major and minor dietary plants; summarize the currently available data on the bioavailability, metabolism, activities, and modes of action of ecdysteroids, mainly

20E, in mammalian systems; and discuss the current and potential impact of this class of compound on the human diet.

For those readers who are not already acquainted with it, the website "Ecdybase" (Lafont et al. 2002), which incorporates several updatable databases associated with various aspects of the ecdysteroid literature, provides a good companion reference to this chapter. Ecdybase grew out of *The Ecdysone Handbook* (Lafont and Wilson 1996). It provides structures, spectral data, and references for all the published phyto-, zoo-, and mycoecdysteroid analogues, biological activity data, and literature surveys of the occurrence of ecdysteroids in plant and non-arthropod species and the effects and uses of exogenous ecdysteroids in animals and plants. To save space in this chapter, rather than referencing many original articles in the primary literature, Ecdybase will be cited, and the original literature can be found there.

33.2 Dietary Phytoecdysteroids

33.2.1 Bioactive Constituents

33.2.1.1 Distribution of Ecdysteroids in the Plant World

Somewhere in the region of 6000 species of plants have been assessed for the presence or absence of ecdysteroids, which accounts for about only 2% of the total number of terrestrial plant species. Additionally, one must bear in mind that studies have used different methods (ranging from bioassays detecting positive extracts to unambiguous spectroscopic identification of specific analogues by NMR or crystallography) differing in their selectivity and sensitivity. Also, different portions of the plants at different stages of development have been assessed. However, the most extensive study has been the Exeter Survey which assessed whether ecdysteroids are present in dormant seeds of ca. 5000 plant species by means of standardized microextraction methodology followed by an ecdysteroid-responsive cell-based bioassay (testing for 20E-like biological activity) and sensitive ecdysteroid-specific radioimmunoassays (testing for chemical similarity to E) (Dinan 1995b). Phytoecdysteroids have been detected in specific members of the ferns, gymnosperms, monocots, and dicots (Lafont 1998). Although phytoecdysteroid-containing species are more common in some genera than in others, where enough species in a genus have been examined, positive species have been found. Also, there is no correlation between species which contain high levels of ecdysteroids and the occurrence of ecdysteroids in other members of the same genus or family, as demonstrated by the rarity of ecdysteroid-containing species in the Asteraceae in general, but the presence of species like *Serratula coronata* and *Leuzea carthamoides*, which contain significantly high levels, in this family. However, if enough species within a family (e.g., the former Chenopodiaceae [now included in the Amaranthaceae]: Dinan et al. 1998) or large genus (e.g., *Silene*; Zibareva et al. 2003) are examined, a pattern in relation to taxonomic structure does begin to emerge, suggesting that the presence and profile of ecdysteroids have chemotaxonomic significance.

33.2.1.2 Distribution of Ecdysteroids Within Plants

Phytoecdysteroids are dynamic molecules within plants; they are not necessarily accumulated where they are biosynthesized; and they demonstrated seasonal variations in where they are located in the plant and how they are metabolized, sometimes resulting in different profiles in different organs at different stages of development. As would be expected of secondary compounds which represent a considerable investment of the plant's resources, phytoecdysteroids seem to be judiciously distributed to enhance the survival of the plant from one year to the next or from one generation to the next in the case of annual plants. Thus, annual cycling of ecdysteroids has been observed in herbaceous biennials or perennials (Lafont et al. 1991), and the association of high levels of ecdysteroids with new growth and/or flower- and seed-producing structures has been observed in several species. Thus, in spinach and quinoa, two annual species of dietary significance, the highest ecdysteroid levels are associated with young shoots, young leaves, flowers, and seeds (Dinan 1992a, b, 1995b).

33.2.1.3 Ecdysteroid Profiles

Ecdysteroid profiles in phytoecdysteroid-containing plants vary from simple, where one analogue prevails (Fig. 2), through moderate, where one to three major analogues are present alongside a "cocktail" of minor analogues, to complex where a mixture of analogues is present and none dominates or where the mixture varies considerably in composition and total ecdysteroid content depending on the ecotype,

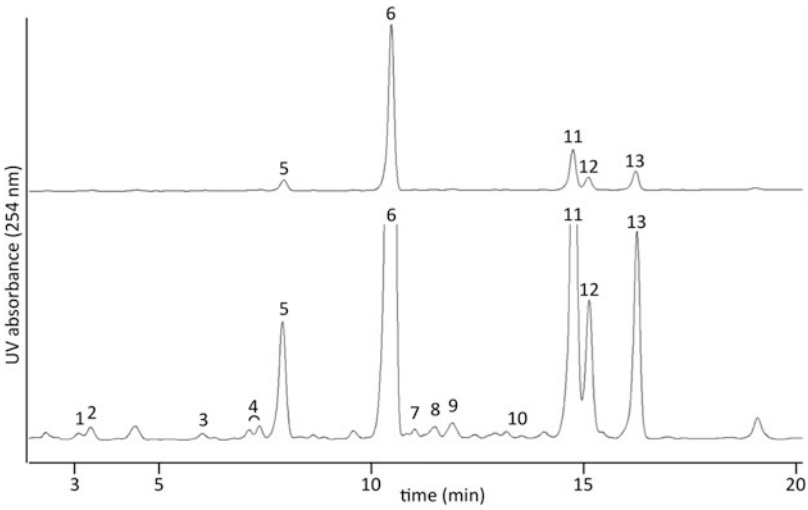

Fig. 2 Chromatograms showing the ecdysteroid cocktail present in a crude extract of *Cyanotis* sp. roots. (1) Dihydrorubrosterone (17β-OH); (2) dihydrorubrosterone (17α-OH); (3) turkesterone; (4) 20,26-dihydroxyecdysone (25*R* + 25*S* isomers); (5) rubrosterone; (6) 20E; (7) inokosterone; (8) 2-deoxy-20,26-dihydroxyecdyone; (9) poststerone; (10) stachysterone B; (11) 20E 3-acetate; (12) ajugasterone C; (13) 20E 2-acetate. The lower panel was recorded with a tenfold increase in sensitivity when compared to the upper panel

growing conditions, or even the individual plant. One presumes that these various profiles are linked to the "strategy" that helps the plant to defend itself from invertebrate predation, but there is no conclusive proof for this. Whatever the distribution and profile of secondary compounds within a plant, it is relatively easy to speculate on a rationale for it, but obtaining substantiating evidence for it requires painstaking study of phytoecdysteroid levels, the generation of plant lines accumulating high and low levels of ecdysteroids, and observations of the effects on the appropriate predator species.

33.2.1.4 Major Analogues

Ecdysteroids are polyhydroxylated steroids which can exist in the free steroid form or as polar or nonpolar conjugates. The structural limits of what constitutes an ecdysteroid are not fully defined, but the majority possesses a 14α-hydroxy-6-keto-7-ene functional group (giving rise to a characteristic UV absorption at 242 nm [ε = 12,400 L/mol/cm] in methanol) with A/B-*cis* ring fusion and *trans* B/C- and C/D junctions. The number of C atoms in unconjugated ecdysteroids depends on which phytosterol they are derived from (C_{27}, C_{28}, or C_{29}; see below) or the extent of side-chain cleavage during biosynthesis (C_{24}, C_{21}, C_{19}). Hydroxyl groups may be located at various positions around the molecule and vary in their stereochemistry, but the most frequently encountered locations are 2β, 3β, 14α, 20*R*, 22*R*, and 25, as found in 20E, the natural arthropod steroid hormone. 20E is the most commonly encountered phytoecdysteroid as it is present in the vast majority of phytoecdysteroid-containing species. 20E is normally also the most predominant ecdysteroid present in a species, frequently accounting for >50% of the total ecdysteroid. Other commonly encountered analogues are polypodine B, pterosterone, turkesterone, and makisterone A (Fig. 3).

33.2.1.5 Range of Analogues and Potential Number

The structural features which categorize a molecule as an ecdysteroid have not been definitively defined, and the approach currently used by most workers in the field is that a molecule should possess several of the chemical features of the archetypal ecdysteroid 20E, i.e., steroid nucleus, polyhydroxylation, 7-en-6-one functional group, A/B-*cis*-ring junction, and C/D-*trans*-ring junction. However, it has been proposed that the spectrum of ecdysteroid analogues can be divided into "true ecdysteroids," which possess a 5β-H, a 14α-hydroxyl group, and a 7-en-6-one chromophore (regardless of whether they possess ecdysteroid biological activity), and "ecdysteroid-related compounds," which do not fulfill all these criteria (Lafont and Horn 1989). A standardized system of abbreviations has been proposed for common ecdysteroids (Lafont et al. 1993).

As more analogues were identified, it became apparent that the range of analogues result from a combination of a number of biochemical changes (hydroxylations, oxidations, reductions, isomerizations, conjugation reactions, etc.) on a basic structure ([5β-H]14α-hydroxy-6-oxo-7-ene cholestane). Over 500 ecdysteroid analogues have been identified, of which 400 have been identified in plants, and more are continuing to be isolated; considering the possible permutations of the possible

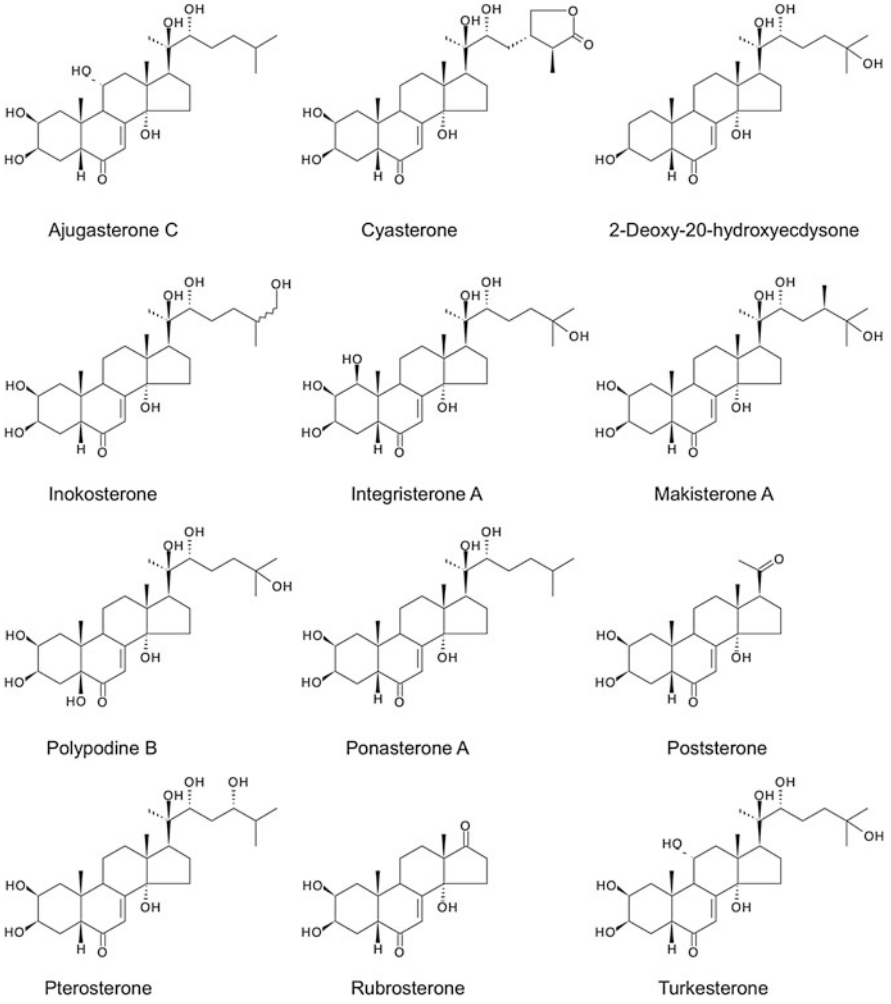

Fig. 3 Structures of significant phytoecdysteroids. See Ecdybase for structures of all published ecdysteroids

biochemical changes involved allows a theoretical calculation that over 1000 analogues could ultimately be identified (Dinan 2001).

33.2.1.6 Biosynthesis in Plants

While considerable progress has recently been made in elucidating the later stages of ecdysteroid biosynthesis from dietary cholesterol or a related sterol in insects and other arthropods, understanding of the biosynthetic pathway(s) and the regulation in plants is still largely unknown. The current state of knowledge has been reviewed (Fujimoto et al. 2000, 2015; Dinan 2001). Very briefly, plants are capable of

generating sterols and steroids de novo by the cytosolic mevalonic acid (MVA) or plastidial methylerythritol phosphate (MEP) routes, although the mevalonate pathway seems to predominate in the biosynthesis of ecdysteroids. Synthesis from cholesterol gives rise to C_{27}-ecdysteroids, while the presence of an alkyl (methyl, methenyl, ethyl, ethenyl) group at C-24 of the sterol gives rise to the corresponding C_{28} and C_{29} ecdysteroids. The wide structural diversity of phytoecdysteroids and the existence of complex cocktails of analogues in many species of phytoecdysteroid-containing plants suggest that there is a lower specificity of the terminal steps (hydroxylases, etc.) of the pathway (after a common intermediate which already contains the 14α-hydroxy-6-oxo-7-ene group) which enables the production of multiple ecdysteroid analogues through permutations in the sequence of the various possible modifications. Thus, the later stages of phytoecdysteroid biosynthesis are probably not a linear pathway but rather a network of steps where differential regulation of the amounts and activities of particular enzymes determines which analogues are generated and their relative amounts in the profile. This would be advantageous for the plant in avoiding a lot of genetic redundancy associated with many specific enzymes and also providing a flexibility to redirect synthesis through differential regulation to new analogues should phytophagous predators become tolerant to previously predominant analogues. The wide taxonomic diversity of plants which accumulate significant amounts of ecdysteroids (see Ecdybase) and the presence of individual plants which accumulate albeit low levels of ecdysteroids in species which are regarded as ecdysteroid-negative (Dinan et al. 2001) imply that most, if not all, plants retain the genetic capacity to generate ecdysteroids, but that the activity of the biosynthetic pathway(s) is downregulated in ecdysteroid-negative plants.

33.2.2 Bioavailability and Metabolism in Mammals

Studies on the pharmacokinetics and metabolism of ecdysteroids in mammals have been sporadic, and our understanding of this important area is far from complete. The early studies (Hikino et al. 1972; Lafont et al. 1988) demonstrated that, in mice, 20E and E were excreted rapidly (within 24 h) and faster after i.p. injection than after ingestion, with the main excretion route being the feces. Ecdysteroids transiently increase in the plasma and liver after administration and then accumulate in the intestine within 1–2 h. Metabolism is initially very limited until the ecdysteroid reaches the large intestine, where gut bacteria dehydroxylate the molecule at C-14, reduce the 6-keto group and bring about C-20/C-22-side-chain cleavage and an enterohepatic cycle (as for bile acids) results in glucuronide conjugation in the liver, transport in the bile and deconjugation in the large intestine. The enterohepatic cycle results in an increasingly complex pattern of metabolites of the administered ecdysteroid and also helps to maintain low but biologically significant levels of ecdysteroids in the plasma. The metabolism of E is simpler, since the absence of a C-20/C-22 diol precludes side-chain cleavage, such that 14-deoxyecdysone is the major metabolite (Girault et al. 1988). 20E is metabolized to 14-deoxy-20-

hydroxyecdysone, 6α-hydroxy-20E, 6α-hydroxy-14-deoxy-20E, poststerone, 14-deoxy-poststerone and 20,26-dihydroxyecdysone as major metabolites (Foucault et al., in preparation). Further metabolites can occur by relocation of the B-ring double bond (Kumpun et al. 2011b) Fig. 4). It is currently not clear if observed biological effects are attributable only to the administered ecdysteroid or, in part, to the metabolites which are generated. Some of the metabolic transformations mentioned above have also been observed in calf urine after the oral administration of 20E (Destrez et al. 2009).

Studies on the pharmacokinetics and metabolism in humans are restricted as it is not possible to use radioactively labelled tracer molecules. Four pharmacokinetic studies have been carried out. Simon and Koolman (1989) used an ecdysteroid-specific immunoassay to monitor urinary excretion after oral ingestion of 15 mg 20E and found that significant immunoreactive material (peaking at ca. 0.5 μM) was excreted in the first 8 h but continues to be excreted at low levels until 24 h (Simon 1988). Brandt (2003) used RIA and HPLC-MS to monitor the excretion of a single oral dose of 20 mg 20E which showed a urinary peak after 3–4 h with a total of 1–2 mg ecdysteroid being recovered in the urine. One of the metabolites was tentatively identified as 14-deoxy-20-hydroxyecdysone. Bolduc (2008) found that only 5 mg 20E was recovered in the urine after the ingestion of 434 mg 20E.

The bioavailability of 20E in mammals is low (<1%), while that of poststerone is somewhat higher (19%). This is in accord with the druggability scores (ADMET Traffic Light Scores; Lobell et al. 2006) for these compounds based on features dependent on their structures and polarities (MW, LogP, polar surface area, number

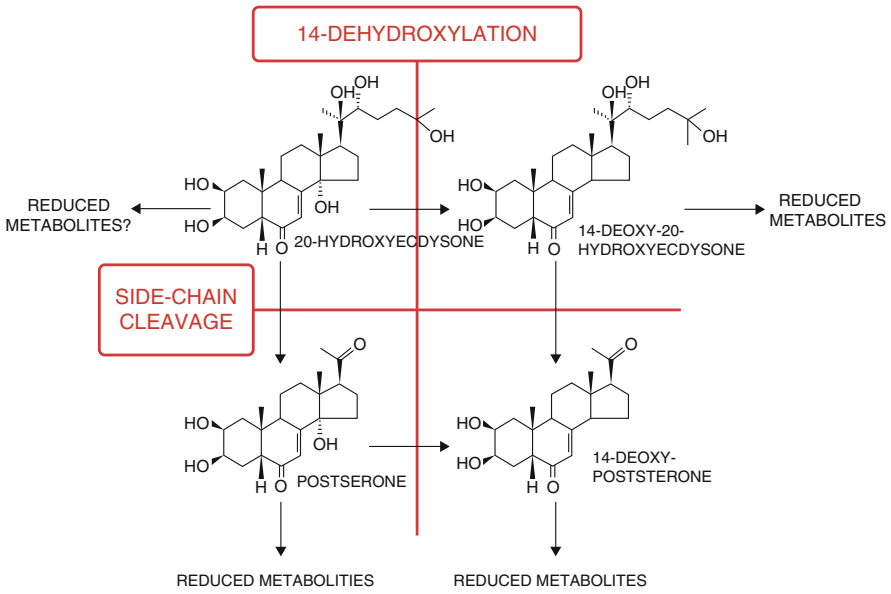

Fig. 4 Metabolism of 20E in mice. (Modified after Kumpun et al. 2011b)

of rotatable bonds, numbers of H-bond donors and acceptors), which gives 20E a score of 3 ("amber"; poor to moderate bioavailability), while Post has a score of 0 ("green"; good bioavailability). This is an aspect requiring attention since the consumption of relatively large amounts of 20E is required to bring about the necessary plasma concentrations required for the induction of biological responses in mammals.

33.2.3 Bioactivities of Ecdysteroids in Mammalian Systems

33.2.3.1 General

The literature up to 2009 has been extensively reviewed previously (Sláma and Lafont 1995; Lafont 1997; Lafont and Dinan 2003, 2009; Dinan and Lafont 2006; Báthori et al. 2008; Dinan 2009). Briefly, in the period after the initial identification of ecdysone and 20-hydroxyecdysone in the mid-1960s, it was considered that ecdysteroids might be used as insect pest control agents, and as a consequence studies were undertaken to obtain toxicological and safety data for mammals exposed to these agents, revealing that they are nontoxic and that they stimulated protein synthesis (summarized below). The next phase, up to about 1984, involved important studies indicating a wide diversity of essentially positive biological activities (anabolic, adaptogenic, wound healing, antidiabetic, hepatoprotective, anti-inflammatory activities, etc.), which were largely published in Russian and unfortunately mainly ignored in the USA and Europe. It was when it became apparent in the mid-1980s that ecdysteroids, in the form of the "Russian Secret," were being used in the belief that they improved the performances of sportsmen and women by increasing muscle mass, stamina, recovery from injury, and mental attitude that the pharmaceutical and medical interest in these molecules started to rapidly increase, such that it is now fair to say that this is now the main focus of ecdysteroid research around the world. Another important factor has made a significant contribution to these advances, and that is the current availability of large amounts of 20E at reasonable cost; in the mid-1970s, 20E was available only in limited amounts and costs at least USD 2000/g, whereas it can now be purchased for USD 1000/kg, which has made clinical trials with this compound feasible.

Since 2010, investigations of the effects of ecdysteroids (essentially 20E) on mammals have concentrated on four main areas: increase of muscle mass, reduction of body fat, antidiabetic effect, and adaptogenic effects.

33.2.3.2 Diabetes

Several plant species (*Ajuga iva*, *A. turkestanica*, *Achyranthes bidentata*) used in traditional medicines for diabetes contain significant levels of ecdysteroids (Lafont 2012). It has been shown that pure 20E significantly reduces hyperglycemia in three rodent models of diabetes at doses of 0.1–10 mg/kg and modifies enzyme levels in the liver such that less glucose would be released from glycogen and more glucose would be converted to lipids. 20E increases glucose consumption by insulin-resistant HepG2 (hepatocyte) cells in a dose- and time-dependent way with a maximal

effect being reached at 5 μM (Chen et al. 2006), which appears to be mediated by an increase in the Glut-4 glucose transporter activity (summarized in Lafont 2012). Kizelsztein et al. (2009) showed, using rat H4IIE hepatoma cells, that 20E stimulates PI3K to phosphorylate/activate Akt, which in turn stimulates the translocation of Glut-4 to cell membranes and thereby facilitating glucose uptake. Further, 20E reduces transcription of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, thus reducing the formation of glucose by release from glycogen and by gluconeogenesis, respectively. 20E also stimulates glucose use by peripheral tissues and increases glycogen content (Syrov et al. 1997), not only of the liver but also in the heart and skeletal muscle (Syrov et al. 1975).

33.2.3.3 Adaptogens

Adaptogens are metabolic regulators present in extracts of certain plants (e.g., *Bryonia alba*, *Eleutherococcus senticosus*, *Rhodiola rosea*, *Schisandra chinensis*) which increase the ability of an organism to adapt to physical, chemical, or biological environmental factors ("stressors") and to avoid damage from such factors by favoring maintenance of homeostasis. Thus, adaptogens help an organism to survive and cope with situations of intense or prolonged stress and to enhance work capacity/mental performance over a longer period, in contrast to stimulants which provide a short-term increase in capacity followed by a rapid decline in performance to below that of the pre-stimulated state (Panossian et al. 1999). The difference seems to derive from adaptogens providing a more general metabolic stimulation of the cells and organs of the organism (involving stimulation of RNA and protein synthesis and ATP production) involving non-specific and specific mechanisms, whereas stimulants act on more specific sites in the CNS which, after a short time, results in exhaustion of the supply of stimulatory neurotransmitter substances. Extracts of the active plants are more effective than pure compounds isolated from the same species, which is in accord with general stimulation through multiple mechanisms, rather than a mode of action involving a single biochemical target. Progress is starting to be made in the elucidation of the complex biochemical modes of action of adaptogens (Panossian 2017), but much remains to be clarified. Some of the plants proposed as adaptogens (*Leuzea carthamoides*, *Tinospora cordifolia*; Panossian and Wikman 2005) are known to be ecdysteroid-containing. *L. carthamoides* is known as maral root, after the maral deer, which has been observed to dig out and consume the rhizomes (containing ca. 1% of the dry weight as 20E) of the plant at the beginning of spring, which improves their overwintered condition remarkably. Consequently, in Eastern Europe, the roots are harvested, dried, and powdered to prepare an adaptogenic tonic for humans.

33.2.3.4 Body Fat

Foucault et al. (2012, 2014) conducted experiments using a diet-induced mouse obesity model, where feeding a high-fat diet rapidly results in the mice becoming obese, but when simultaneously fed 20E at 5 or 10 mg/kg, there was a much lower fat increase which was not a consequence of lower food intake. Adipocytes from treated mice were reduced in size, but not in number and tissue inflammation, and the

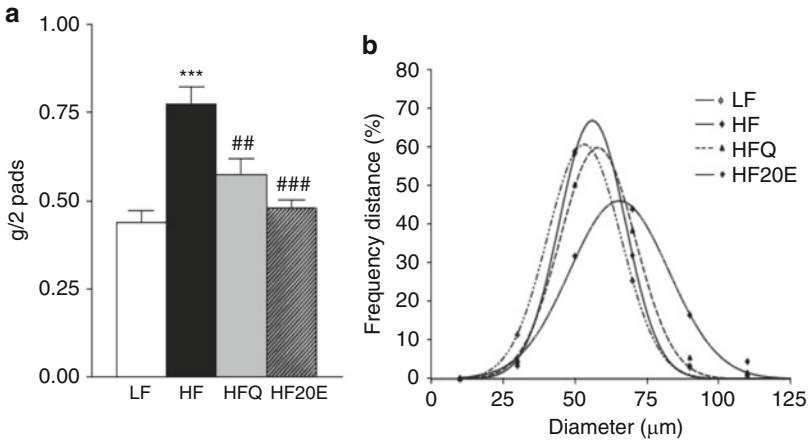

Fig. 5 Body growth and adipose tissue cellularity. Data are expressed as mean ± s.e.m. ($n = 12$ per group). (**a**) Epididymal adipose tissue weight; (**b**) distribution of epididymal adipose cell diameters. $***P < 0.001$, when compared to LF group; $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$, when compared to HF group. *20E* 20-hydroxyecdysone, *HF* high-fat diet, *LF* low-fat diet, *Q* quinoa extract enriched in 20E. (After Foucault et al. 2012, reproduced with permission)

levels of cytokines involved in adipocyte growth and differentiation were reduced (Fig. 5). Adiposity was also reduced in ovariectomized rats treated with 20E (Seidlova-Wuttke et al. 2010a). The mechanism of action of this response has not yet been investigated.

33.2.3.5 Muscle Mass

The mouse C_2C_{12} myocyte cell line, when stimulated with 20E or turkesterone, increases the incorporation of [^{3}H]leucine into protein rapidly (maximum incorporation after 2 h) and in a dose-dependent manner. The mechanism of action involves the PI3K/Akt system and an influx of Ca^{2+} ions. The effects of 20E were suppressed by inhibitors of GPCRs, phospholipase C, and PI3K. In parallel in vivo experiments, it was shown that treatment of rats with 20E for 28 days significantly increased their grip strength (Gorelick-Feldman et al. 2008; 2010). Male Wistar rats receiving 20E (5 mg/kg body weight) by subcutaneous injection into the left thigh for 8 days showed a significant increase in body mass, and the *soleus* and *extensor digitorum longus* muscles were significantly enlarged on both sides with increases in the number of muscle fiber nuclei, implying activation of the satellite cells (Tóth et al. 2008). In conjunction with the effect on adipocytes described above, 20E enhances lean body mass.

Previous studies had shown that insulin and IGF-1 stimulation of muscle protein is mediated by PI3K/Akt activation of mTORC1 assembly and signal transduction (Fig. 6). Anthony et al. (2015) could find no evidence for enhanced phosphorylation of Akt, mTOR, or 4E-BP1 in rat muscle or liver after oral administration of 20E. Bioavailability of the 20E was poor in the rats, and even using an excipient

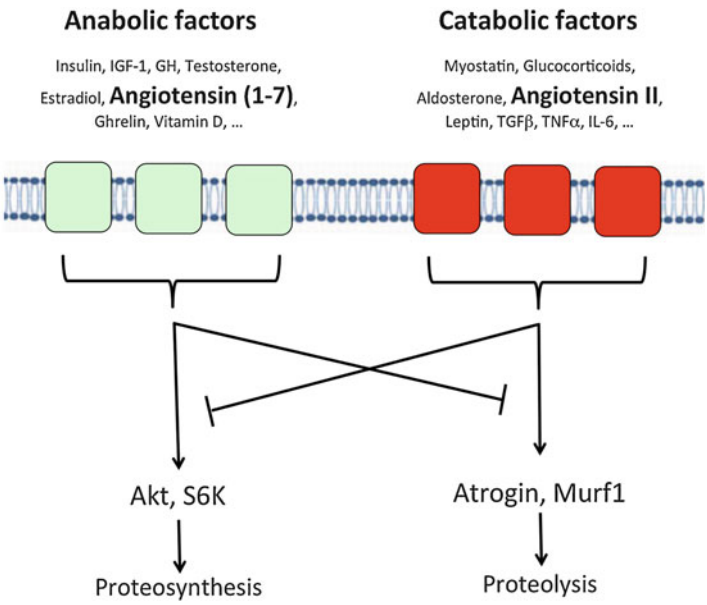

Fig. 6 Major actors and signalling pathways coordinating muscle protein balance. Red and green square represent various membrane receptors

(Labrasol) to enhance 20E bioavailability did not result in increased phosphorylation of the signalling components. These data appear to be in conflict with those of Gorelick-Feldman et al. (2008, 2010), but the route of 20E application was different. Unfortunately, it is not known if the rats used by Anthony et al. (2015) would have demonstrated muscular hypertrophy, as the animals were sacrificed after 4 h or less after gavage with 20E.

33.2.3.6 The Nutritional and Medical Applications of Phytoecdysteroids

In view of the broad spectrum of positive biological activities of ecdysteroids in mammals and their low toxicity, ecdysteroids have nutritional and pharmaceutical potential as food supplements and medicines. While these two areas possess some aspects in common, they do differ fundamentally in several important ways:

Food supplements: Preparations sold as food supplements normally consist of an extract enriched in the active component(s) in a largely undefined matrix which should be nontoxic; the registration procedure is considerably facilitated if the concentrated extract derives from a recognized food plant. The daily intake of the active substances is low (<100 mg/day), although the amount of the plant extract may be considerably higher (1–2 g), depending on the concentration of the active compound in the plant matrix. The activity may be a result of the active components alone, or derive from additive or synergistic interactions between the identified active substances and components of the matrix. The claimed activities of food supplements should be supported by scientific evidence, but it is not necessary that

the mode of action should be fully understood. Food supplements are made available to the general public (i.e., to a more or less healthy population), to improve/maintain general well-being, improve diet and digestion, and enhance the ability to cope with stress. Nutritional clinical trials may be performed on potential food supplements based on dietary plants, but there is no legal requirement for this.

Quinoa, an extract of *Chenopodium quinoa* seed enriched in ecdysteroids, was investigated as a food supplement to promote muscle mass and fat loss. It is perhaps instructive to briefly describe the developments in this area as a fairly typical example of its kind. Initially, certain ecdysteroid-containing food plant species (*Ajuga iva*, *Chenopodium quinoa*, *Spinacia oleracea*; Bakrim et al. 2008, 2014; Kumpun et al. 2011a) were assessed for their ecdysteroid distributions, levels, and profiles, which led to quinoa seed being selected for further development. The initial studies had investigated the ecdysteroid contents of seeds from various sources and also the ecdysteroid contents in bran and flour after various milling techniques, which identified quinoa bran as a more concentrated source of 20E (Kumpun et al. 2011a). The next stage was to develop a simple and cost-effective extraction procedure which was amenable to scale-up. Quinoa bran (450 kg; ca. 0.08% w/w 20E) was mixed with water (4500 L) and shaken for 2 h at room temperature and then filtered. The filtrate was heated to coagulate protein, filtered again, and then concentrated tenfold, before being applied to an Amberlite FPX66 resin column, to which the ecdysteroids adhere and were eluted with ethanol. The eluate was spray-dried to yield a preparation which contained 2.4% (w/w) 20E (Foucault 2012). An alternative approach based on leaching ecdysteroids from quinoa seeds has also been described (Graf et al. 2014).

The 20E-enriched extract (quinoa) when fed to mice prevented significantly the development of obesity in the same way as an equivalent amount of 20E, which could be attributed to effects on energy metabolism such that more glucose was oxidized, lipogenesis decreased, and less dietary lipid uptake was observed (Fig. 5: Foucault et al. 2012, 2014). On the basis of these very encouraging results, a clinical trial of quinoa, involving 118 overweight and obese adult humans, compared the effects of 1.6 g quinoa/day (containing 40 mg 20E) to 1.6 g of placebo in a 12-week controlled double-blind trial separated into two 6-week periods, a weight-loss phase (restricted calorific intake) followed by a weight-loss maintenance phase. Patients were monitored for body weight, obesity parameters, dietary intake, and various relevant biochemical parameters at 0, 6, and 12 weeks. Although the data indicated a tendency to prevent body weight and fat mass regain when compared to placebo, the differences were not statistically significant to the required degree, which may be attributable to the relatively low dosage of 20E in the quinoa and reflect one of the difficulties in preparing food supplements: the balance between the amount of active ingredient (20E) and the daily amount of the preparation (quinoa) that the patient can be expected/is willing to take. However, a statistically significant improvement of insulin sensitivity could be observed in this study.

Medications: The regulatory regime is much stricter for compounds intended as medicines. These are aimed for use by a smaller population suffering from a recognized and defined medical condition. The preparation consists of an essentially

pure active compound in a fully defined formulation, and the dosage is normally considerably higher than those encountered in food supplements, and this dosage has to be defined and shown to be effective and safe in relation to the age and gender of the target group. With regard to plant-derived active compounds, the source of the compound, its purification, its formulation, and its route of application must be fully defined. The drug will also have to successfully undergo preclinical and clinical trials before it receives authorization from the national regulatory agency:

Preclinical: In vitro and in vivo (nonhuman animal) studies to obtain information about efficacy, toxicity, pharmacokinetics, and mode of action.
Phase 0: Studies performed on a small number [10–15] people to obtain preliminary information on the bioavailability and half-life of the drug in humans.
Phase I: The drug is tested on healthy volunteers (20–100) with ascending doses to assess safety. Pharmacokinetics and pharmacodynamics are also determined.
Phase II: Tested on patients (100–300) suffering from the specific disease at the therapeutic dose to assess efficacy and identify side effects.
Phase III: The therapeutic dose is tested in a large-scale, multicenter trial (300–3000 patients) to assess efficacy, effectiveness, and safety.

The sequence of clinical trials are increasingly costly, and only a relatively low percentage of drugs entering Phase I trials successfully complete Phase III and obtain regulatory approval. There is also a Phase IV trial which monitors the performance of the drug once it becomes available for prescription. It used to be the case that large pharmaceutical companies funded the necessary research and development from initial lead compound to marketed medicine, but increasingly the identification of leads and initial trials are being undertaken by smaller innovative companies specializing in particular therapeutic areas or approaches, with the larger companies becoming involved, through licensing or purchase, to guide and finance a promising drug through the Phase III trial.

Although many patents attest to the pharmaceutical potential of ecdysteroids in human medicine, no drugs based on ecdysteroids have yet received regulatory approval.

33.2.3.7 Mode(s) of Action

The modes of action of 20E at responding mammalian cells have not been fully clarified, and several models are being pursued. The models are not mutually exclusive, and it is possible that they could operate simultaneously or in different tissues. Given the pleiotropic effects described for 20E on mammalian cells, it seems possible that it operates through several signalling pathways, which could be more or less tissue-specific.

Gorelick-Feldman et al. (2010) clearly established that 20E acts through a membrane GPCR receptor, and this has been confirmed using albumin-bound 20E which cannot cross cell membranes (Raynal et al. 2015).

The renin-angiotensin system (RAS) is strongly implicated in maintaining muscle function, and one of the peptide products of this system, angiotensin II, targets

skeletal muscle cells via the AT1R receptor and has been implicated in the development of sarcopenia (Yoshida et al. 2013), both directly through AT1R which results in increased resistance to insulin and IGF-1 and indirectly by increased production of myostatin, glucocorticoids, TNF-α, and IL-6. Consequently, ACE inhibitors (which inhibit the production of angiotensin II) have found some application in the treatment of sarcopenia. Another actor of the RAS is angiotensin 1-7 which has been identified as the natural ligand for another GPCR, Mas, activation of which has been hypothesized to enhance protein synthesis in muscle cells. Thus, RAS would have a "harmful arm" acting through AT1R where activation of the receptor enhances proteolysis, but this can be counteracted by a "protective arm," acting through Mas, where activation of the receptor enhances protein synthesis. Thus, according to this hypothesis, protection against muscle wasting can be achieved by reducing activation of AT1R, or activation of Mas, or a combination of the two. There are reasons to believe that activation of Mas would be more effective at stimulating muscle anabolism and reducing adipose tissue than an ATIR antagonist, and has fewer side effects than ACE inhibitors.

Mas receptor is expressed in many tissues, and its activation in different tissues (e.g., heart, kidney, CNS) may evoke protective effects (Höcht et al. 2009) (Fig. 7). Activation of Mas by 20E could explain the anabolic effects of 20E on muscle cells (Raynal et al. 2015).

Parr et al. (2014) have put forward an alternative model for the mode of action of 20E in bringing about the hypertrophy of mammalian skeletal muscle cells which involves interaction of the steroid with nuclear estrogen receptor-β (ERβ). Using C_2C_{12} cells, they observed that both 20E (1 μM) and E2 (1nM) enhanced myotube diameter. Co-treatment with the antiestrogen ZK antagonized the hypertrophy

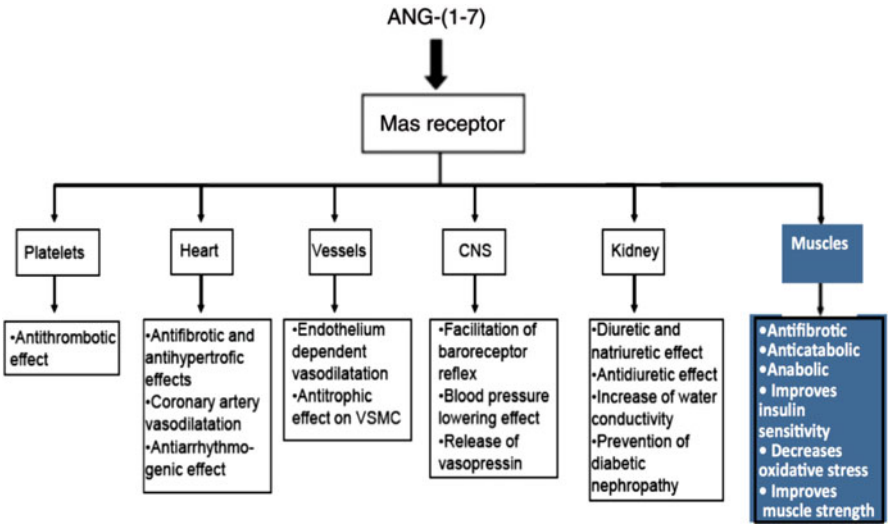

Fig. 7 The multiple targets of Mas receptor. (Adapted from Höcht et al. 2009)

brought about not only by E2 but also by 20E, indicating that E2 and 20E share a common mode of action. The authors next showed that a reporter gene under the control of an estrogen response element could be activated dose dependently by E2 or 20E interacting with ERα or ERβ and that this activation could be prevented if ZK was also present. Use of the estrogen receptor-specific agonists, ALPHA (for ERα) and BETA (for ERβ), indicated that ERβ mediated the hypertrophy of the C_2C_{12} myotubes, but a note of caution is required because these agonists are only selective at low concentrations and the beta dose-response curve was bell-shaped. Finally, the selective ERβ-antagonist (ANTIBETA) antagonized the effects of E2 and 20E in the C_2C_{12} cells. The authors suggest that, as it had previously been shown that ERβ can modulate Akt phosphorylation, this could be the link by which 20E brings about hypertrophy in the C_2C_{12} cells. In silico docking studies suggest that 20E can interact with the ligand-binding domains of ERα and ERβ but has the potential for stronger interaction with ERβ (Parr et al. 2015). However, we may question the exact nature of the receptor involved in the observed effects, because all binding studies performed with nuclear receptors ERα and ERβ were negative (Báthori et al. 2008), and truncated membrane-bound forms of ER receptors have been described that may display different ligand specificity (Schreihofer et al. 2018). Finally, we wish to mention the recent article by Sobrino et al. (2017). These authors provide evidence that in primary human umbilical vein endothelial cells (HUVEC) cultures, E2 acts via membrane-bound ER to bring about NO-dependent vasodilation and they show that this effect also requires Mas activation, as it is abolished by a Mas inhibitor. Although this a different system to the muscle cells above, it might perhaps give an indication of how both 20E and E2 can bring about muscle hypertrophy.

33.2.3.8 Potency and Structure-Activity Relationships

The availability of so many ecdysteroid analogues, which often differ by just one biochemical change from other known analogues, is a considerable resource for SAR studies and has been used to advantage to characterize the ecdysteroid nuclear receptor in insects (Dinan and Hormann 2005). However, no such comparable extensive study has yet been performed for an ecdysteroid-induced effect in a mammalian system. There are several reasons for this, but most are related to the fact that the biological systems are not yet well characterized enough. The potency of a molecule depends on its affinity for its target, rates of metabolism and excretion, sequestration, and the nature of the cellular response. Most responses to ecdysteroids in mammalian systems appear to require concentrations of 20E in the 0.1–10 μM range, but this does not mean very much unless one has a good understanding of the mode of action and metabolism, both of which might vary from system to system and are only now starting to be elucidated.

The earliest SAR for ecdysteroids in a mammalian system was for the stimulation of protein synthesis in mouse liver (Uchiyama and Otaka 1974), where 11 ecdysteroids were compared at 50 μg/100 g body weight. Nine of the ecdysteroids (20E, inokosterone, cyasterone, ponasterone A, pterosterone, shidasterone, lemmasterone, ponasteroside A, and polypodine B), all possessing a C-20/C-22-diol, were active in a time-dependent manner, as was rubrosterone, which is a C19 ecdysteroid lacking the side chain. Ecdysone (E), however, showed no activity.

When four of the ecdysteroids (20E, Ino, Cyast, and Rub) were tested at 5 μg/100 g body weight, only cyasterone remained active. Rubrosterone may have a different mode of action to the other ecdysteroids, as it has been suggested that its structure is close to the androgenic compound androsta-4-en-3,17-dione (Báthori et al. 2008).

Gorelick-Feldman et al. (2008) compared four ecdysteroids (20E, Turk, PolB, and PoA) for their ability to stimulate the incorporation of [^{3}H]leucine into protein in mouse C_2C_{12} cells in culture which had been differentiated into muscle myotubes. All four were active in a dose-dependent manner, with 20E and Turk appearing to be more active (EC_{50} = ca. 0.04 μM) than PolB and PoA (EC_{50} = ca. 0.1 μM).

Báthori et al. (2008) laudably collated the diverse findings in the early literature (published in Russian) on the anabolic activities of 23 ecdysteroid analogues in 6 types of rodent-based assays (not all analogues had been assessed in all of the assays). This brought out the importance of the hydroxyls at 2β, 3β, 11α, and 20*R* for the stimulation of amino acid incorporation into mouse liver proteins, such that turkesterone and its tetra-acetylated derivative had the highest activity. However, the relative potencies of 20E and Turk depend on the biological system used (cf. Gorelick-Feldman et al. 2008), indicating that factors other than target sensitivity (e.g., bioavailability, metabolism, sequestration, clearance) also affect overall potency.

An initial investigation of the relationship between ecdysteroid structure and the promotion of stamina has been assessed for swimming duration until exhaustion in mice (Mamadalieva et al. 2008; Table 1). Notably, all ten pure ecdysteroid analogues tested promoted the duration of swimming by 10–40% after receiving 5 mg/kg body weight per os 1 h before the test, while siverinol (a mixture of ecdysteroids extracted from aerial portions of *Silene viridiflora*), also at 5 mg/kg body weight, enhanced swimming duration by just under 50%, implying synergistic interaction between the ecdysteroids in the mixture. The most active pure analogues were 20E, Turk, silenoside A, and silenoside D, but not enough analogues were tested to develop a meaningful SAR. The ecdysteroids were more effective than a couple of adaptogenic plant extracts and some synthetic compounds (an androgenic compound and a psychotropic substance) used in higher amounts. Only Fenamin (an anti-inflammatory agent) prolonged swimming duration more than the ecdysteroids (Table 1).

While these studies provide amenable assays and demonstrate the fundamental characteristics of time and dose dependency, they are too preliminary in terms of validation, reproducibility of the response, and range of tested analogues to provide anything other than a glimpse of what the SAR might be.

33.2.4 Potential Benefits

33.2.4.1 Aging

Loss of muscle mass and strength is a normal aspect of aging, starting from about the age of 40 and with a loss of ca. 1% per annum, rising to 2% beyond the age of 70. Although inevitable for the vast majority of people, individuals can slow down the process by regular exercise and a good, balanced diet. Unfortunately, the decline can

Table 1 Influence of ecdysteroids (and other substances) on swimming duration until exhaustion of mice bearing a load (5% of body weight). Animals were treated 1 h before starting the swimming period. Male mice (18–20 g) received treatments per os; pure ecdysteroids or siverinol (5 mg/kg b.w.), natural product mixtures as *Eleutherococcus* (Siberian ginseng) extract containing a complex mixture of phenolic compounds (0.2 mL/20 g b.w) or Saparal (a mixture of triterpene glycosides extracted from the roots of *Aralia manchurica*; 20 mg/kg b.w.), and synthetic compounds as Nerobol (methandrostenolone, a non-aromatizable anabolic androgen, 10 mg/kg b.w.), Bemithyl (2-ethylthiobenzimidazole hydrobromide, a psychotropic substance, 50 mg/kg b.w.) and Fenamin (mefenamic acid, a non-steroidal anti-inflammatory analgesic, 10 mg/kg b.w.). Data from Mamadalieva et al. (2008)

Experimental conditions	Duration of swimming (min)	% relative to control
Control	34.0 ± 1.2	100
Ecdysone	38.7 ± 0.7	114
2-Deoxyecdysone	37.0 ± 1.2	109
Integristerone A	39.3 ± 0.4	116
Polypodine B	41.0 ± 0.6	121
20-Hydroxyecdysone	45.6 ± 0.9	134
Turkesterone	46.5 ± 0.6	137
Silenoside A	48.8 ± 1.0	143
Silenoside C	43.0 ± 0.8	126
Silenoside D	46.7 ± 0.9	137
Silenoside E	41.3 ± 0.8	121
Siverinol[a]	50.7 ± 0.8	149
Eleutherococcus extract	39.3 ± 1.0	116
Saparal	41.2 ± 1.3	121
Bemithyl	44.7 ± 1.8	131
Nerobol	40.0 ± 0.9	118
Fenamin	64.8 ± 2.5	191

[a]A partially purified ecdysteroid-containing methanol extract of *Silene viridiflora* (Caryophyllaceae)

become a vicious circle, since declining muscle strength results in ever less inclination to remain active, with many only recognizing the problem once it has become established, and it is perhaps too late to pursue a more active lifestyle. Biochemically, the rate of skeletal muscle protein synthesis declines with aging, owing largely to decreased activation of the PI3K/Akt/mTORC1 signalling pathway (Zwetsloot et al. 2014). Hence, the potential attractiveness of mild anabolic agents, such as the ecdysteroids, which activate this signalling pathway through a G-protein-coupled receptor, and, unlike the androgenic anabolic steroids, and does not require a concomitant exercise regime for effect and does not produce unpleasant physical and psychological side effects. However promising ecdysteroids may seem as "exercise mimetics," they need yet to be thoroughly tested by means of double-blind, placebo-controlled clinical trials to assess for efficacy and safety, especially as it is a medication which will need to be taken for the rest of life. Also, it cannot be expected that agents like ecdysteroids would be able to reverse age-related muscle loss, but they could slow the progress of the decline.

33.2.4.2 Sarcopenia

Under certain circumstances in a proportion of the aging population, the rate of muscle mass and function loss is greater and causes a general deterioration of physical state. Muscle fibers atrophy and the muscles become fibrotic (contain regions of collagen). This process is accompanied by motoneuron death. Obesity can aggravate sarcopenia, by decreasing functional capacities further. Sarcopenia enhances the risk of falls and fractures and is associated with greater incidence of dementia. Once muscle mass/strength is lost past the age of ca. 60, it is increasingly difficult to reverse the process, and beyond the age of 70, it is virtually impossible to do so, hence the importance of a healthy, active life from at least middle age and preferably throughout life. It is estimated that 30% of the US population between 65 and 79 is affected by sarcopenia, rising to 50% among the over 80s. This translates as 14.8 million sufferers among a population of 308 million (Baumgartner et al. 1998).

33.2.4.3 Cachexia

Cachexia is the rapid muscle and adipose tissue wasting associated with loss of weight, fatigue, weakness, and loss of appetite occurring in conjunction with certain conditions, e.g., cancer, multiple sclerosis, rheumatoid arthritis, tuberculosis, or anorexia nervosa. Loss of >5% of body weight over 6 months indicates that cachexia may be present. Ca. 50% of cancer patients suffer from cachexia, and it significantly increases the risk of mortality. The mechanism(s) by which the above diseases cause cachexia are not well understood, and control of the condition is currently poor. It is plausible that ecdysteroids might help to slow loss of body mass associated with cachexia, but there is no current proof of this. A recent study has implicated activation of the Mas receptor (see Sect. 2.3.7 above) in the attenuation of muscle wasting in cachexia (Murphy et al. 2018).

33.2.4.4 Duchenne Muscular Dystrophy

DMD is a rare recessive X-linked inherited genetic disorder which affects about 1 in 3,500 boys (ca. 30,000 afflicted in Europe). It involves mutations of various degrees of severity which affect the expression of the dystrophin gene, the product of which is a major component of the cytoskeleton in muscle cells and is important in maintaining muscle cell structure and providing resistance to damage. Those affected undergo a gradual physical decline from about the age of 5, becoming increasingly weaker and less independently mobile, with death normally ensuing by the age of 30–40. A number of therapeutic strategies are currently being assessed for DMD, but no cure is presently available. It is envisaged that ecdysteroids might slow down the muscular decline or support the maintenance of muscle mass after gene therapy.

33.2.4.5 Type 2 Diabetes (Metabolic Syndrome)

The inexorable rise of the incidence of type 2 diabetes around the world (rising from 108 million sufferers in 1980 to 422 million in 2014, corresponding to 8.5% of the adult population; www.who.int/news-room/fact-sheets/detail/diabetes) is a major health and social problem. The characteristic symptom is elevated and imperfectly

regulated blood glucose levels, but this can arise by a number of mechanisms (reduced/inadequate insulin production, reduced sensitivity to insulin, etc.), so that in reality there is a syndrome of metabolic diseases of differing severity (Ahlqvist et al. 2018). Although genetic predisposition plays a role, it is the modern lifestyle which combines poor diet (excessive calories, processed foods, low amounts of fresh fruit and vegetables) with limited regular exercise, resulting in large sections of the population becoming overweight or even obese, which is strongly associated with the development of type 2 diabetes, which normally begins with lethargy or the need to drink larger volumes of water, but, if not adequately treated, can go on, after two to three decades, to result in blindness, loss of sensory feeling and poor blood flow in extremities (especially the feet), amputations, and early death. The onset used to be in late middle age, but in addition to the rapidly increasing incidence, the age of onset is dropping, such that the incidence among children is now very concerning. Early detection, before nerve and blood vessel damage has become too established, coupled with lifestyle/dietary changes and medication (if appropriate) is paramount to controlling blood glucose levels and prolonging the patient's active life. The effects of ecdysteroids on carbohydrate, protein, and lipid metabolism in mammals are particularly interesting in this respect. Many studies have now shown that ecdysteroids, while not affecting blood glucose levels in healthy subjects (Uchiyama and Yoshida 1974), reduce hyperglycemia. Again, these promising preclinical studies need to be supported by thorough double-blind clinical studies.

33.2.4.6 Anabolic Activity

Ecdysteroids (mainly 20E, but also turkesterone) are already extensively used by certain sportsmen, bodybuilders, bouncers, etc. to increase muscle mass and stamina. 11α-Hydroxy ecdysteroids, such as turkesterone, are claimed to have higher anabolic activity than 20E (Syrov 2000). The absence of the side effects (long-term muscle degeneration, virilization, roid (as it is a contraction of steroid) rage, etc.) characteristic of androgenic-anabolic steroids is an attractive feature of ecdysteroids for these user groups. Further, the anti-inflammatory effects may aid recovery from injuries. Many unregulated products are sold largely through the Internet, and they contain purified ecdysteroids or partially purified plant extracts (mainly from *Pfaffia glomerata* or *Cyanotis arachnoidea* [for 20E] and *Ajuga turkestanica* [for Turk]), often combined with other supposedly anabolic substances (protein, essential amino acids, creatine, etc.), but the composition and batch-to-batch reproducibility are uncertain. Their consumption is not supervised and does not follow a scientifically verified regimen; rather the users tend to follow the empirical advice of other users. As with all other unregulated pharmaceuticals, the safety of such preparations can be questioned. Regulation and standardization would benefit all.

33.2.4.7 Osteoporosis

Menopause in women is often accompanied by osteoporosis, where bone strength is lost and the incidence of fractures increases. Ecdysteroid-containing plants (*Achyranthes bidentata*, *Tinospora cordifolia*) have been traditionally used in Chinese and Indian medicines for the treatment of osteoporosis. Ecdysteroid-

containing plant extracts or pure 20E have an osteoprotective effect in ovariectomized rats without any effect on the uterus or mammary glands (Seidlova-Wuttke et al. 2010b). 20E stimulates the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (Lafont 2012). Ecdysteroids also show promise to counteract secondary osteoporosis brought about when glucocorticoids are used in medication (Dai et al. 2017).

33.2.4.8 Anti-stress

The adaptogenic properties of ecdysteroids have been recognized in Eastern Europe and Russia for several decades (Panossian and Wikman 2005), and there is scope to expand this knowledge to increase resilience to the modern lifestyle by reducing the impacts of physical and mental stress, increase stamina, and increase resistance to infections. In this sense preparations of the water-soluble ecdysteroid might be consumed on an occasional basis as tonics and as components of teas or infusions. It seems that adaptogens cease to be effective if they are consumed on a daily basis for too long a period (a week to a few months, depending on the adaptogen and the performance required; Panossian and Wikman 2005), so it is to be expected that the ecdysteroid preparations would be consumed when fatigued and stressed, rather than as a "daily tonic." Thus, such preparations might help in people with seasonal affective disorder (SAD), loss of libido, loss of appetite, chronic fatigue, age-associated lethargy, sport-associated weariness, poor sleep patterns, and alcohol or narcotic withdrawal (Panossian et al. 1999)

33.2.4.9 Alzheimer's Disease

There are numerous reports indicating that ecdysteroids have potential as neuroprotective agents, in the treatment of neurological diseases, and the improvement of memory and learning. Thus, it is perhaps not surprising that their effects in model systems for Alzheimer's disease are beginning to be assessed. Alzheimer's disease is a neurodegenerative disease and is believed to account for 60–70% of cases of dementia. As humans are living longer, the incidence of Alzheimer's is increasing, such that there were 30 million sufferers worldwide in 2015. In the USA, in 2000, the incidence was 1.6% in the 64–74 age group, 19% in the 75–84 age group, and 42% in the >84 year olds, at a cost of $100 billion per year (Wikipedia: Alzheimer's disease). The risk factors are predominantly (70%) genetic, but environmental factors also play a role (e.g., smoking, pollution, poor diet, lack of physical or mental exercise). There is currently no cure, but certain treatments and lifestyle choices may delay onset or the development of symptoms. Progress in the search for effective medication has been hampered by the first symptoms (usually short-term memory loss) becoming apparent many years (perhaps decades) after the biochemical defects have been initiated, with the result that clinical trials of possible treatments have been performed on patients at a too advanced stage of the disease. Several hypotheses have been proposed for the underlying cause of the disease, of which the two major ones are the amyloid and tau hypotheses, which are, respectively, associated with the deposition and accumulation of insoluble extracellular amyloid-β plaques and the hyperphosphorylation of tau protein, which normally

stabilizes microtubules, resulting in neurofibrillary tangles in nerve cell bodies, bringing about cell death. Studies involving ecdysteroids have concerned the amyloid hypothesis. The N-terminal fragment of amyloid precursor protein (N-APP) and the adjacent amyloid-β proteins (Aβ; 39–42 amino acids in length) are aberrantly cleaved from the large amyloid precursor proteins (APP; several isoforms) by β-secretase 1 and γ-secretase (Chakraborty and Basu 2017). According to one version of the hypothesis, $A\beta_{1-42}$ initiates the plaques seen in the brains of Alzheimer's patients, and soluble forms bind to neuron cell-surface receptors and disrupt neuronal communication, while, according to a more recent version of the hypothesis, N-APP binds to neuronal receptor DR6 inducing self-destruction of the cell by apoptosis. An initial study (Yang et al. 2003) considered the ability of 20E to prevent aggregation of $A\beta_{1-42}$ into fibrils (precursors of the plaques) and found that 100 μM 20E prevented fibril formation in PBS and that neuronal cell viability consequent on treatment with $A\beta_{1-42}$ was increased in a dose-dependent manner by 20–100 μM 20E. In an independent study (Yang et al. 2010), involving six ecdysteroid analogues isolated from *Klaseopsis chinensis*, five of the analogues were found to enhance or decrease fibril formation, but, oddly, 20E was without effect at 50 μM. In a more recent study (Chakraborty and Basu 2017), 20E (20 μM) was found to prevent $A\beta_{25-35}$ (an analogue of $A\beta_{1-42}$, which also forms fibrils, but perhaps not with the same kinetics or affinity) aggregation in vitro. This study also looked at the effect of 20E on β-secretase 1 (BACE 1) and provides experimental evidence that 20E both binds to BACE 1 with a relatively high affinity (Kd = 1.75 μM) and prevents its catalytical activity, which were supported by in silico molecular docking studies which indicated that 20E binds to the active cavity of the enzyme, but does not interact directly with the amino acid residues responsible for catalysis. 20E also prevents Aβ-induced mitochondrial-dependent apoptosis in cultured human neuroblastoma cells (Xu et al. 2018). Thus, ecdysteroids appear to have potential to target several possible sites in the early development of Alzheimer's disease. However, studies on rats after i.p. injections of ecdysteroids show that although 20E diacetonide will cross the blood-brain barrier, 20E itself does not (Kalász et al. 2017).

33.2.5 Application in Food

33.2.5.1 Dietary Intake

Findeisen (2004) undertook a survey of 115 food items for the presence of ecdysteroids as detected by enzyme immunoassay, using the ecdysteroid-specific DBL2 antiserum. In addition to various fruits, vegetables, and nuts, she also tested a selection of meats, fish, ready meals, herbs and spices, dairy products, and drinks. Although low levels of immune-positive material were detected in most food samples, only fresh spinach (*Spinacia oleracea*; Amaranthaceae) contained a significant level (2210 μg 20E eq./g dw [0.22%]; 185 μg/g fw), with the next highest concentration being found in a sample of common mushrooms (*Agaricus bisporus*) at only 95 μg 20E eq./g dw; 8.5 μg/g fw. On the basis of the data obtained, it was

possible to estimate the intake of ecdysteroids associated with four main courses with meat or fish, or fast food, or vegetarian, all of which were calculated to contain between 10 and 66 μg 20E equivalents/meal, although none of these meals contained spinach and any losses during preparation were not allowed for. However, the data clearly indicate that the average dietary intake of ecdysteroids, at least in the Western European diet, is rather low, probably only very occasionally exceeding 100 μg/day. Also, these approximate calculations do not allow for the effects of cooking. Thus, Simon (1988) found that cooked spinach contained low levels of immunoreactive ecdysteroids (0.7 nmol ecdysone eq./kg = 0.3 ng E eq./g wet weight), indicating that most is leached out or destroyed during cooking of the leaves and stalks.

Around the world, the diversity of food plants is considerably greater than the range customarily consumed in the current Western diet. It is estimated that in total 20,000 species of plants are consumed to a greater or lesser extent by humans (https://pfaf.org/user/edibleuses.aspx). Most of these correspond to wild plants, and not to cultivars which have been optimized for yield and uniformity, but may also have reduced resistance to predators and diseases owing to lower levels of secondary compounds. However, 90% of the calorific intake from plant sources across the world is accounted for by just 33 crops (Table 2). Where these plants have been assessed for ecdysteroid content (not necessarily the consumed part), these major crop plants appear to contain little or no detectable ecdysteroids.

In certain parts of the world, the so-called pseudocereals have contributed significantly to the diets of the local populations over many centuries, and several of these have recently been "discovered" by Western societies and valued for their nutritional properties and even regarded as "super foods." Significantly, a high proportion of these crops are members of the Amaranthaceae (Table 3) and contain levels of ecdysteroids similar to those found in spinach; most of the other pseudocereals have not yet been adequately assessed to be able to say whether or not they contain ecdysteroids. A 100 g portion of quinoa would contain ca. 50 mg ecdysteroids (mainly 20E) before preparation and cooking. The extent of retention of ecdysteroids in quinoa grain during the usual preparation and cooking procedures has not been extensively studied, but it is known that immersion in water even at 25 °C results in leaching of about 20% of the ecdysteroid in 24 h (Graf et al. 2014). However, Kumpun et al. (2011a) found that, under normal cooking conditions (boiling for 20 min), >70% of the 20E was retained within the grain after cooking. These two findings together imply that ecdysteroids are present in quinoa grain in two forms, a readily leachable form and a more strongly retained form.

As part of the preparation for this chapter, a database was compiled of the major and minor food plants and the parts of the plant consumed, combined with information on whether any part of the plant had yet been examined for the presence of ecdysteroids and the levels detected (derived from the literature/Ecdybase and the Exeter Survey of ecdysteroid levels in plant seeds). Of the 746 edible plants included, 294 have been in any way examined for the presence of ecdysteroids, and only 17 (5.8%) contained more than 10 μg/g dw (>0.001%): *Amaranthus* spp. (Saeng-ngam et al. 2004; Bespayeva et al. 2012), *Atriplex* spp. (Dinan et al. 1998), *Chenopodium* spp. (Rastrelli et al. 1996; Dinan et al. 1998), *Dioscorea dumetorum*

Table 2 The 33 major world plant crops. (Modified from http://oregonstate.edu/instruct/ccs/330/two/Unit4Notes.htm) and their content of ecdysteroids: (a) sourced from Ecdybase (+ relevant reference); (b) sourced from Exeter Survey

Plant	Latin binomial	Family$	Plant parts eaten	Area harvested (Ha × 10^6)*	Production (tonne × 10^6)*	Ecdysteroids§
Wheat	*Triticum aestivum*	Poaceae	Grain	211	568	[a]–(seeds: Dinan 1995a) [b]Grain: –
Rice	*Oryza sativa*	Poaceae	Grain	146	579	[a]–(plant: Takemoto et al. 1967))/+[0.09§] (leaf: Blackford et al. 1996) [b]Grain: –
Maize	*Zea mays*	Poaceae	Cobs	139	602	[a]–(plant: Devarenne et al. 1995; leaf: Blackford et al. 1996) [b]Kernels: +[0-4§]
Soybean	*Glycine max*	Fabaceae	Seeds	79	180	[a]–(plant: Takemoto et al. 1967; leaf Blackford et al. 1996) [b]Beans: –
Barley	*Hordeum vulgare*	Poaceae	Grain	54	132	[a]–(aerial parts: Clément and Dinan 1991) [b]Grain: –
Sorghum	*Sorghum bicolor*	Poaceae	Grain	42	55	[a]–(leaf: Blackford et al. 1996) [b]Grain: –
Finger millet	*Eleusine coracana*	Poaceae	Grain	37	26	nt [b]Grain: –
Proso millet	*Panicum miliaceum*					[a]–(leaf: Blackford et al. 1996) [b]Grain: –
Pearl millet	*Pennisetum glaucum*					nt
Foxtail millet	*Setaria italica*					[a]–(leaf, stem, root, flower: Dinan et al. 2001) [b]Grain: –
Yellow bristle grass	*Setaria pumila*					nt

Groundnut (peanut)	*Arachis hypogaea*	Fabaceae	Seeds	26	34	[a]+[0.1§] (leaf: Blackford et al. 1996) [b]nuts: –
Mung bean	*Phaseolus aureus*	Fabaceae	Seeds	25	18	[a]–(leaf: Blackford et al. 1996) [b]Beans: –
Runner bean	*Phaseolus coccineus*					[a]–(leaf: Blackford et al. 1996) [b]Beans: +[0.2§]
Lima/butter bean	*Phaseolus lunatus*					nt [b]Beans: –
French bean	*Phaseolus vulgaris*					nt [b]Beans: –
Rapeseed	*Brassica napus*	Brassicaceae	Seed oil	23	33	[a]–(leaf: Blackford et al. 1996) [b]Seed: +[0.4§]
Sugarcane	*Saccharum officinarum*	Poaceae	Cane juice, stems	20	1288	nt [b]Seed: –
Sunflower	*Helianthus annuus*	Asteraceae	Seeds	20	23	[a]–(leaf: Blackford et al. 1996) [b]Seeds: –
Potato	*Solanum tuberosum*	Solanaceae	Tuber	19	308	[a]–(leaf: Blackford and Dinan 1997a, b)
Cassava	*Manihot esculenta*	Euphorbiaceae	Root	17	180	[a]–(leaf: Blackford et al. 1996) [b]Seeds: –
Oats	*Avena sativa*	Poaceae	Grain	13	28	[a]–(Dinan 1995a) bGrain: –
Coconut	*Cocos nucifera*	Arecaceae	Flesh, water	11	49	[a]–(inflorescence: Sreejit 2014)
Oil palm	*Elaeis guineensis*	Arecaceae	Fruit oil	11	136	nt
Chickpea	*Cicer arietinum*	Fabaceae	Seeds	11	8	nt

(continued)

Table 2 (continued)

Plant	Latin binomial	Family$	Plant parts eaten	Area harvested (Ha × 10^6)*	Production (tonne × 10^6)*	Ecdysteroids§
Coffee	*Coffea arabica*	Rubiaceae	Beans	11	8	nt [b]Seeds: –
	Coffea conephora					nt
Rye	*Secale cereale*	Poaceae	Grain	10	21	nt [b]Grain: –
Sweet potato	*Ipomoea batatas*	Convolvulaceae	Tuber	9	141	[a]–(leaf: Blackford et al. 1996)
Cowpea	*Vigna unguiculata*	Fabaceae	Seeds	9	3	nt
Olives	*Olea europaea*	Oleaceae	Fruit	8	15	nt [b]Seeds: –
Grape	*Vitis vinifera*	Vitaceae	Fruit	7	62	[a]–(leaf: Blackford and Dinan 1997b) [b]Seeds: –
Sesame	*Sesamum indicum*	Pedaliaceae	Seeds	7	3	nt [b]Seeds: –

Cocoa	*Theobroma cacao*	Sterculiaceae	Beans	7	3	[a]+[0.08§] (leaf: Blackford et al. 1996)
Sugar beet	*Beta vulgaris* var. *vulgaris*	Amaranthaceae	Root	6	252	[a]–(aerial parts: Clément and Dinan 1991)/ +(?: Báthory et al. 1984) [b]Seeds: –
Pea	*Pisum sativum*	Fabaceae	Seed	6	10	nt [b]Seeds: –
Apple	*Malus pumila*	Rosaceae	Fruit	6	58	nt [b]Seeds: –
Plantain	*Musa sapientum*	Musaceae	Fruit	5	29	nt [b]Seeds: –

++, significant amounts detected; +, low levels detected; –, not detected; *nt*, not tested
$Family name according to APGIV (2016)
§Where values are given they correspond to μg ecdysone equivalents/g dw as determined with the antiserum DBL1 in an ecdysteroid-specific RIA
∗Data for 2002

Table 3 Pseudocereals and the content of ecdysteroids in the grain/seed

Common name	Latin binomial	Family[a]	Plant part assessed	Amount ecdysteroid[b]	Method	References
Purple amaranth	*Amaranthus cruentus*	Amaranthaceae	Plant in flower	0.015% dw as 20E	HPLC	Bespayeva et al. (2012)
			Seeds	0.2 μg E eq/g	RIA	Exeter Survey
Common amaranth	*Amaranthus retroflexus*	Amaranthaceae		nt		Ecdybase
			Seeds	–	RIA	Exeter Survey
Prickly amaranth	*Amaranthus spinosus*	Amaranthaceae	Whole plant	+	Bioassay	Takemoto et al. (1967)
Amaranth	*Amaranthus tricolor*	Amaranthaceae		nt		Ecdybase
			Seeds	–	RIA	Exeter Survey
Slender amaranth	*Amaranthus viridis*	Amaranthaceae	Whole plant	+	Bioassay	Takemoto et al. (1967)
			Leaf			Saeng-ngam et al. (2004)
			Stem	0.7mg20E% dw 0.2mg20E% dw	TLC TLC	
Pitseed goosefoot	*Chenopodium berlandieri*	Amaranthaceae		+		Ecdybase
			Seeds	145 μg E eq/g	RIA	Dinan et al. (1998)
Kaniwa	*Chenopodium pallidicaule*	Amaranthaceae	Seeds	15 μg 20E/g	Isolation	Rastrelli et al. (1996)
Quinoa	*Chenopodium quinoa*	Amaranthaceae	Seeds	612–1292 μg E eq/g	RIA	Dinan et al. (1998)
			Seeds	316–421 μg 20E/g	HPLC	Kumpun et al. (2011a)
			Seeds	419 μg 20E/g	LC/UV/MS	Graf et al. (2014)
Hanza, aizen, boscia	*Boscia senegalensis*	Capparaceae		nt nt		Ecdybase Exeter Survey
Chia	*Salvia hispanica*	Lamiaceae		nt		Ecdybase
			Seeds	–	HPLC/MS	Biophytis (unpublished)
Flax, linseed	*Linum usitatissimum*	Linaceae		nt		Ecdybase
			Seeds	–	HPLC/MS	Biophytis (unpublished)
Breadnut	*Brosimum alicastrum*	Moraceae		nt nt		Ecdybase Exeter Survey

(continued)

Table 3 (continued)

Common name	Latin binomial	Family[a]	Plant part assessed	Amount ecdysteroid[b]	Method	References
Sesame	*Sesamum indicum*	Pedaliaceae		nt		Ecdybase
			Seeds	–	RIA	Exeter Survey
			Seeds	–	HPLC/MS	Biophytis (unpublished)
Buckwheat	*Fagopyrum esculentum*	Polygonaceae	Whole plant	–	Bioassay	Takemoto et al. (1967)

[a]Family name according to APGIV (2016)
[b]Levels given are for intact plant parts and do not allow for changes in content during preparations and/or cooking

(Sautour et al. 2008), *Halimione portulacoides* (Dinan et al. 1998), *Lamium* spp. (Savchenko et al. 2001), *Silene acaulis* (Zibareva et al. 2003), *Spinacia oleracea* (Dinan et al. 1998), and *Vitex donania* (Ochieng et al. 2013). Further, where plants have been assessed, it is often not the portions of the plant which are consumed which have been assessed for ecdysteroids. The database will be added to the Ecdybase website to provide access to the information and in a format which can be updated as further information becomes available.

In view of the limited nature of the data concerning the presence and quantification of ecdysteroids in food plants, it seemed worthwhile to assess a range of staple foods and a wide variety of fruits and vegetables with a specific HPLC-MS/MS method for the quantification of 20E (the most frequently encountered phytoecdysteroid in the plant world) and targeting those parts of the plants which are typically consumed. Of the plant food sources assessed, only spinach leaves/petioles and quinoa grain contained significant amounts of 20E, while virtually all other samples (Table 4) contained no detectable 20E. The spinach sample was found to contain 236 μg 20E/g dw, while a sample of commercial quinoa grain contained 138 μg 20E/g dw, which was reduced to 98 μg/g dw after heating at 100 °C in 10 volumes of water for 15 min (i.e., 32% of the 20E was lost to, and recovered in, the cooking water). The only other sample found to be positive was lemon (*Citrus limon*) fruit at 1.04 μg/g dw, which was at the limit of detection. However, this is interesting in the light of the report of the presence of ecdysteroids in *Citrus medica* (citron; Yin et al. 2015), which is a wild progenitor from which modern lemon and some lime hybrid cultivars are derived (summarized in Velasco and Licciardello 2014). Assessment of fruits of *C. medica* var. *sarcodactylis* (hand of Buddha), *C. maxima* (pomelo), *C. x clementina* (clementine), *C. hystrix* (kaffir lime), or *C. japonica* (kumquat) by HPLC-MS/MS did not detect 20E at or above the limit of detection (Table 4).

A further database has been prepared for the occurrence of ecdysteroids and ecdysteroid-related molecules in edible fungi. The world production of edible mushrooms is not insignificant, being 7,700,000 tonnes in 2011, of which 65%

Table 4 Plant foods (vegetables, fruits, herbs, and spices) tested for the presence of 20-hydroxyecdysone by means of a sensitive and specific HPLC-MS/MS method. Plant materials were purchased from supermarkets, freeze-dried, and powdered, and samples (ca. 100 mg) were extracted with 50% aq. ethanol (1.5 mL) at 75 °C for 2 h. An aliquot (1 mL) was diluted with water (4 mL) and partially purified on a pre-activated RP-SPE (SEPAK; Waters) cartridge, washed with 10% aq. methanol (5 mL), and eluted with 100% methanol (5 mL). Aliquots (5 μL) were subjected to RP-HPLC-MS for quantification of 20E (limit of detection = 50 ng/mL). See the text for a summary of the results

Latin binomial	Family[a]	Common name	Plant parts assessed
Abelmoschus esculentum	Malvaceae	Okra	Fruit
Actinidia arguta	Actinidiaceae	Negri, mini kiwi	Fruit
Actinidia deliciosa	Actinidiaceae	Kiwi fruit	Fruit pulp
Agaricus bisporus	Agaricaceae	Common mushroom	Fruiting bodies
Allium cepa ascalonicum	Amaryllidaceae	Shallot	Bulb
Allium cepa	Amaryllidaceae	Onion	Bulb
Allium porrum	Amaryllidaceae	Leek	Leaf sheath
Allium sativum	Amaryillidaceae	Garlic	Cloves
Ananas comosus	Bromeliaceae	Pineapple	Fruit
Annona cherimola	Annonaceae	Cherimoya, custard apple	Fruit
Apium graveolens	Apiaceae	Celeriac	Swollen hypocotyl
Arachis hypogaea	Fabaceae	Peanut, groundnut, monkey nut	Nut (not shell)
Avena sativa	Poaceae	Oats	Rolled oats
Averrhoa carambola	Oxalidaceae	Starfruit	Fruit
Beta vulgaris vulgaris	Amaranthaceae	Beetroot	Swollen root
Brassica oleracea botrytis	Brassicaceae	Cauliflower	Florets
Brassica oleracea capitata	Brassicaceae	Savoy cabbage	Leaves
Brassica oleracea italica	Brassicaceae	Broccoli	Florets
Brassica rapa var. rapa	Brassicaceae	Turnip	Swollen taproot
Capsicum annuum	Solanaceae	Sweet pepper	Fruit
Carica papaya	Caricaceae	Papaya	Fruit flesh
Carya illinoinensis	Juglandaceae	Pecan	Nuts
Castanea sativa	Fagaceae	Sweet chestnut	Seed (not shell)
Chenopodium quinoa[c]	Amaranthaceae	Quinoa	Grain
Chenopodium quinoa[c]	Amaranthaceae	Quinoa	Cooking water
Chenopodium quinoa[c]	Amaranthaceae	Quinoa	Cooked grain
Cicer arietinum	Fabaceae	Chickpea	Dry seed
Citrus x clementina	Rutaceae	Clementine	Fruit flesh
Citrus hystrix	Rutaceae	Kaffir lime	Whole fruit
Citrus japonica	Rutaceae	Kumquat	Whole fruit

(continued)

Table 4 (continued)

Latin binomial	Family[a]	Common name	Plant parts assessed
Citrus limon[b]	Rutaceae	Lemon	Fruit
Citrus maxima (C. grandis)	Rutaceae	Pomelo	Fruit flesh
Citrus medica var sarcodactylis	Rutaceae	Citron "hand of Buddha"	Fruit
Cocos nucifera	Arecaceae	Coconut	Coconut water
Cocos nucifera	Arecaceae	Coconut	Coconut flesh
Coriandrum sativum	Apiaceae	Coriander	Seeds
Corylus avellana	Betulaceae	Hazelnut, cobnut, filbert	Nut (no shell)
Cucumis metuliferus	Cucurbitaceae	Kiwano, horned melon, melano	Fruit flesh and seeds
Cucurbita moschata	Cucurbitaceae	Butternut squash	Fruit
Cucurbita pepo	Cucurbitaceae	Courgette, zucchini	Fruit
Cuminum cyminum	Apiaceae	Cumin	Seeds
Cydonia oblonga	Rosaceae	Quince	Fruit flesh
Cyphomandria betacea (Solanum betaceum)	Solanaceae	Tamarillo	Whole fruit
Daucus carota	Apiaceae	Carrot	Root
Diospyros kaki	Ebenaceae	Asian persimmon	Fruit
Elettaria cardamomum	Zingiberaceae	Green cardamom	Pods
Ficus carica	Moraceae	Fig	Fruit
Foeniculum vulgare	Apiaceae	Florence fennel	Bulb and stem
Fragaria x ananassa	Rosaceae	Strawberry	Fruit
Garcinia mangostana	Clusiaceae	Purple mangosteen	Fruit pulp and seed
Helianthus tuberosus	Asteraceae	Jerusalem artichoke	Tuber
Hylocereus megalanthus	Cactaceae	Yellow pitaya	Fruit flesh
Ipomoea batatas	Convolvulaceae	Sweet potato	Tuber
Juglans regia	Juglandaceae	Walnut	Kernels
Linum usitatissimum	Linaceae	Common flax, linseed	Seeds
Lycopersicon esculentum	Solanaceae	Tomato	Fruit
Malus domestica	Rosaceae	Apple	Flesh
Mangifera indica	Anacardiaceae	Mango	Fruit flesh
Musa x paradisiaca	Musaceae	Banana	Flesh
Myristica fragrans	Myristicaceae	Nutmeg	Ground seeds
Nephelium lappaceum	Sapindaceae	Rambutan	Fruit flesh
Nephelium lappaceum	Sapindaceae	Rambutan	Fruit coat
Nephelium lappaceum	Sapindaceae	Rambutan	Seeds
Nigella sativa	Ranunculaceae	Love-in-a-mist, black cumin	Seeds
Opuntia ficus-indica	Cactaceae	Prickly pear, Barbary fig	Fruit
Oryza sativa	Poaceae	Brown rice	Grain

(continued)

Table 4 (continued)

Latin binomial	Family[a]	Common name	Plant parts assessed
Papaver sp.	Papaveraceae	Poppy	Seeds
Passiflora edulis	Passifloraceae	Passion fruit	Fruit pulp and seeds
Pastinaca sativa	Apiaceae	Parsnip	Root
Persea americana	Lauraceae	Avocado	Flesh
Persea americana	Lauraceae	Avocado	Kernel
Phaseolus vulgaris	Fabaceae	French bean	Immature pods
Pimpinella anisum	Apiaceae	Anise	Seeds
Pinus pinea	Pinaceae	Pine nuts	Kernels
Prunus dulcis	Rosaceae	Almond	Shelled nut
Punica granatum	Lythraceae	Pomegranate	Fruit
Pyrus communis	Rosaceae	European pear	Fruit flesh
Raphanus raphanistrum sativus	Brassicaceae	Radish	Swollen root
Ribes rubrum	Grossulariaceae	Redcurrant	Fruit
Robinia pseudoacacia	Fabaceae	Black locust	Seed
Rubus fruticosus	Rosaceae	Blackberry	Fruit
Rubus idaeus	Rosaceae	Raspberry	Fruit
Sechium edule	Cucurbitaceae	Chayote, mirliton squash	Fruit
Sesamum indicum	Pedaliaceae	Sesame	Seeds
Sinapis alba	Brassicaceae	White mustard	Seeds
Solanum melongena	Solanaceae	Aubergine, eggplant	Fruit
Solanum tuberosum	Solanaceae	Potato	Tuber
Spinacia oleracea[c]	Amaranthaceae	Spinach	Leaves and stalks
Tamarindus indica	Fabaceae	Tamarind	Fruit pulp
Tamarindus indica	Fabaceae	Tamarind	Seeds
Tamarindus indica	Fabaceae	Tamarind	Shell
Trigonella foenum-graecum	Fabaceae	Fenugreek	Seeds
Triticum aestivum	Poaceae	Wheat	Flour (wholemeal)
Vaccinium macrocarpon	Ericaceae	Cranberry	Fruit
Vaccinium myrtillus	Ericaceae	Blueberry	Fruit
Vigna radiata	Fabaceae	Mung bean	Seeds
Vitis vinifera	Lamiaceae	Grape	Fruit
Zingiber officinale	Zingiberaceae	Ginger	Rhizome

[a]Family name according to APGIV (2016)
[b]Positive for 20E at the LOD (see text)
[c]Positive for 20E (see text)

were produced in China followed by 10% in Italy and 5% in the USA (Wikipedia: edible mushrooms). Mycoecdysteroids (reviewed in Kovganko 1999) have been much less extensively investigated than phytoecdysteroids, but it appears from the

data currently available that the levels found in fruiting bodies are generally low in comparison to those in phytoecdysteroid-containing plants and that a different range of structural modifications are found in fungi than in plants, such that 20E is often not the predominant ecdysteroid in a mycoecdysteroid profile and the ergostane-type ecdysteroid analogues are often related to cerevisterol ([22*E*,24*R*]-3β,5α,6β-trihydroxyergosta-7,22-diene). Since mushroom fruiting bodies are susceptible to attack by invertebrate pests (predominantly, but not exclusively, soil nematodes and dipteran sciarid [fungus gnats], phorid [humpbacked flies], and cedid [midges] flies; Singh and Sharma 2016), it is possible that mycoecdysteroids also serve a defensive function. No studies have been performed on the biosynthesis of mycoecdysteroids, so it is currently not possible to say if they are endogenously synthesized and taken up from the substrate or a precursor is taken up and modified by the fungus. An extract of the database, focusing on the edible mushrooms which have been investigated for ecdysteroids and ecdysteroid-related compounds, is presented in Table 5. It is clear that currently very few species of edible mushrooms have been examined for the presence of ecdysteroids and ecdysteroid-related compounds. The most extensively studies species is Polyporus umbellatus, which is not only edible but it is used extensively in traditional Chinese, Japanese and Indian medicines, primarily to treat oedema and as a diuretic, but also for other conditions, and without toxicity or side-effects (Zhao 2013).

33.2.5.2 Dietary Supplements

At the present time, 20E or extracts of ecdysteroid-containing plants (particularly *Pfaffia glomerata*; Brazilian ginseng) are incorporated into protein supplements aimed at body builders and sportsmen with the stated claim of enhancing the anabolic effect. With the growing interest in the potential of ecdysteroids to delay or reduce the impacts of age-related or genetically determined muscle wasting conditions or, more generally, to counteract various stresses as adaptogens, it is to be expected that range of ecdysteroid-supplemented foods will increase significantly in the near future and that they will be of interest to wider sections of society.

33.2.6 Safety: Toxicity and Side Effects

33.2.6.1 Data on Low Toxicity and Absence of Negative Side Effects

Ogawa et al. (1974) determined LD_{50}s for ingested 20E or inokosterone in mice of >9 g/kg and an LD_{50}s of 6.4 g/kg and 7.8 g/kg for i.p.-injected 20E and inokosterone, respectively. Thus, ecdysteroids are regarded as nontoxic to mammals. Also, no effects were seen after the administration of these two ecdysteroids to bullfrogs or rabbits. The two ecdysteroids did not have sex hormonal or, interestingly in the context of more recent studies, anti-inflammatory or anabolic effects in rats.

33.2.6.2 Ecdysteroids and Cancer

El Mofty et al. (1987, 1994) have claimed that E is carcinogenic to toads and mice, but the certainty of these findings has been questioned (Sláma and Lafont 1995;

Table 5 Edible fungal species for which the fruiting bodies have been assessed for the presence or absence of ecdysteroids or ecdysteroid-related compounds. Where possible, the quantities determined and the methods used are also given. This table summarizes and expands the information provided in the reviews by Kovganko (1999) and Mamadalieva (2013)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
Agaricus blazei (A. subrufescens)	Agaricaceae	Almond mushroom, himematsutake	Commercially cultivated	Cerevisterol (2.3 mg from 5 kg fw), 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene (3.4 mg), 3β,5α,6β,9α-tetrahydroxyergosta-7,22-diene (6.3 mg), 3β,5α,9α-trihydroxyergosta-7,22-diene-6-one (5.7 mg)	Kawagishi et al. (1988); isolation and spectroscopic identification
Agaricus bisporus	Agaricaceae	Common mushroom	Commercially cultivated	Chestnut: 0.32 μg 20E eq./g dw	Findeisen (2004); ecdysteroid EIA (DBL2 antiserum)
				White 1: 20.1 20E eq. μg/g dw	
				White 2: 95.2 20E eq. μg/g dw	
				–	Biophytis (unpublished); HPLC-MS/MS for 20E
Amanita muscaria	Amanitaceae	Fly agaric	Edible only after cooking	–	Dinan (unpublished); RIA for ecdysteroids (DBL1 antiserum)

Amanita rubescens	Amanitaceae	Blusher	Edible only after cooking	–	Dinan (unpublished); RIA for ecdysteroids (DBL1 antiserum)
Boletus edulis	Boletaceae	Edible boletus	Commercially harvested from the wild	–	Dinan (unpublished); RIA for ecdysteroids (DBL1 antiserum)
Calvatia cyathiformis	Lycoperdaceae	Purple-spored puffball	Edible wild species	Calvasterone ([6,6′*R*]-biergosta-4,4′,7,7′,22,22′-hexaene-3,3′-dione)	Kawahara et al. (1993); isolation and spectroscopic identification
				Cyathisterone (ergosta-7,22-diene-3,6-dione; 30 mg from 4 kg fw)), cyathisterol (8β-hydroxyergost-4,6,22-trien-3-one; 80 mg), ergosta-4,7,22-triene-3,6-dione (300 mg), ergosta-4,6,8(14),22-tetraen-3-one (200 mg)	Kawahara et al. (1994); isolation and spectroscopic identification
				Calvasterol A (14α-hydroxyergosta-4,7,9,22-teraen-3,6-dione; 10 mg from 4 kg fw), calvasterol B (9α,14α-dihydroxyergosta-4,7,22-trien-3,6-dione; 15 mg from 4 kg fw),	Kawahara et al. (1995); isolation and spectroscopic identification

(continued)

Table 5 (continued)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
Flammulina velutipes	Physalacriaceae	Enoki mushroom, velvet shank, winter fungus	Commercially cultivated	5α,8α-Epidioxy-(24*S*)-ergost-6-en-3β-ol (0.9 mg from 4.1 kg fw), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (0.2 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (0.5 mg), (24*S*)-ergost-7-ene-3β,5α,6β-triol (0.2 mg)	Yaoita et al. (1998); isolation and spectroscopic identification
Ganoderma lucidum	Ganodermataceae	Lingzhi	Commercially exploited for nutritional and dietary products	Ergosta-4,7,22-triene-3,6-dione (314 mg from 5.32 kg fw)	Hirotani et al. (1987); isolation and spectroscopic identification
				6α-Hydroxyergosta-4,7,22-trien-3-one, 6β-hydroxyergosta-4,7,22-trien-3-one	Nishitoba et al. (1988); isolation and spectroscopic identification

Grifola frondosa	Meripilaceae	Maitake, hen-of-the-woods	Commercially harvested from the wild	(22*E*,24*R*)-Ergosta-7,9(11),22-triene-3β,5α,6β-triol (2.7 mg from 20 kg fw), (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6α-triol (1.5 mg), 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (1.1 mg), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (4.3 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α-triol (1.0 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (8.2 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β,9α-tetrol (4.7 mg)	Ishizuka et al. (1997); isolation and spectroscopic identification
Hericium erinaceus	Hericiaceae	Lion's mane, monkey head	Commercially cultivated/ commercially harvested from the wild/used as food additive	Cerevisterol (73 mg from 3.8 kg dw), cerevisterol-3-glucoside (29 mg), 3β,5α,9α-trihydroxyergosta-7,22-dien-6-one (32 mg)	Takaishi et al. (1991); isolation and spectroscopic identification
Hypsizygus tessellatus (H. marmoreus)	Tricholomataceae	Beech mushroom	Commercially cultivated/ edible only after cooking	5α,9α-Epidioxy-3β-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (8.4 mg from 4.3 kg fw), 5α,9α-epidioxy-3β-hydroxy-(24*S*)-ergost-7-en-6-one (4.2 mg), 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one (1.0 mg), 3β,5α,9α,14α-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-	Yaoita et al. (1998); isolation and spectroscopic identification

(continued)

Table 5 (continued)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
				6-one (1.4 mg), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (3.6 mg), (24*S*)-ergost-7-ene-3β,5α,6β-triol (12.8 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (38 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (1.4 mg), ergosta-7,24(28)-diene-3β,5α,6β-triol (0.3 mg)	
Lentinula edodes (Lentinus edodes)	Marasmiaceae	Shiitake mushroom	Commercially cultivated	3β,5α,9α-Trihydroxy-(22*E*,24*R*)-23-methylergosta-7,22-dien-6-one (0.5 mg from 4.7 kg fw), 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one (0.9 mg), 3β,5α,9α,14α-tetrahydroxy-22*E*,24*R*)-ergosta-7,22-dien-6-one (1.0 mg), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22,-dien-6-one (17.1 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (1.3 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6a-triol (0.6 mg), (22*E*,24*R*)-ergosta-7,9(11),22-triene-3β,5α,6α-triol (0.6 mg),	Yaoita et al. (1998); isolation and spectroscopic identification

				(22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol (3.3 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β,9α-tetrol (6.2 mg)	
Pholiota microspora (P. nameko)	Strophariaceae	Nameko, butterscotch mushroom	Usually eaten cooked	3β,5α,9α-Trihydroxy-(24*S*)-ergost-7-en-6-one (1.0 mg from 3.0 kg fw), 4 (0.7 mg), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (3.0 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (0.6 mg), (24*S*)-ergost-7-ene-3β,5α,6β-triol (0.2 mg)	Yaoita et al. (1998); isolation and spectroscopic identification
Pleurotus ostreatus	Pleurotaceae	Oyster mushroom	Commercially cultivated/ commercially harvested from the wild	5α,9α-Epidioxy-3β-hydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (1.9 mg from 2.7 kg fw), 3β,5α,9α-trihydroxy-(24*S*)-ergost-7-en-6-one (0.6 mg), 3β,5α,9α,14α-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (0.4 mg), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (3.5 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6α,9α-tetrol (0.4 mg), 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (0.2 mg), (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-	Yaoita et al. (1998); isolation and spectroscopic identification

(continued)

Table 5 (continued)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
				triol (13.4 mg), (24*S*)-ergost-7-ene-3β,5α,6β-triol (0.2 mg)	
				0.07 μg 20E eq./g dw	Findeisen (2004); EIA of ecdysteroids (DBL2 antiserum)
Polyporus umbellatus	Polyporaceae	Lumpy bracket, umbrella polypore	Only young fruiting bodies are edible	Polyporusterones A (300 mg from 100 kg dw), B (300 mg), C (26 mg), D (8 mg), E (22 mg), F (10 mg) and G (10 mg)	Ohsawa et al. (1992); isolation and spectroscopic identification
				(20*S*,22*R*,24*R*)-16,22-Epoxy-3β,14α,23β,25-tetrahydroxyergost-7-en-6-one (4 mg from 39.2 kg dw), (23*R*,24*R*,25*R*)-23,26-epoxy-3β,14α,21α,22α-Tetrahydroxyergost-7-en-6-one (8 mg)	Zhou et al. (2007); isolation and spectroscopic identification
				Polyporoids A (2 mg from 1 kg dw), B (7 mg) and C (3 mg), polyporusterones A	Sun and Yasukawa (2008); isolation and

				(10 mg), B (14 mg), C (18 mg) and G (3 mg), ergosta-7,22-diene-3β,5α,6β-triol (3 mg)	spectroscopic identification
				(22*E*,24*R*)-Ergosta-6-en-3β,5α,6β-triol (0–0.040 mg/g dw), (22*E*,24*R*)-ergosta-6,22-dien-3β,5α,6β-triol (0–0.073 mg/g dw), polyporusterone F (trace – 0.087 mg/g dw)	Zhao et al. (2009); HPLC-MS
Tapinella atrotomentosa (Paxillus atrotomentosus)	Tapinellaceae	Velvet roll-rim, velvet-footed pax	Only young fruiting bodies are edible	Paxillosterone (356 mg from 6.65 kg fw), paxillosterone 20,22-p-hydroxybenzylidene acetal (5.5 mg), atrotosterones A (41 mg), B (3.9 mg) &C (5.5 mg), 25-hydroxyatotosterones A (11.9 mg) & B (4.2 mg), 20E (41 mg)	Vokáč et al. (1998); isolation and spectroscopic identification

Lafont and Dinan 2009) on the basis of the low doses used, relative to the amounts of ecdysteroids expected in the animals' normal diets. Neves et al. (2016) believe that 20-hydroxyecdyonse is genotoxic in a rat bone marrow micronuclei assay and an *Allium cepa* (onion) cell abnormality assay. However, other reports have claimed that ecdysteroids have no effects on tumor cell growth or even possess antitumor activity either alone (Hirono et al. 1969; Lagova and Valueva 1981; Takasaki et al. 1999) or in conjunction with known anticancer agents (Konovalova et al. 2002; Martins et al. 2012, 2015). Studies of this sort should be conducted with ecdysteroids of the highest degree of purity, which may not always have been the case.

33.2.6.3 Ecdysone and Effects on the Kidney

Recently (Lu et al. 2018a, b), it has been suggested that ecdysone (E, which is rarely a major phytoecdysteroid) has detrimental effects on renal function in mice at a dose of 6 μg/g/d, bringing about glomerular injury and proteinuria. The authors propose that E acts as a mimic of aldosterone, activating the mineralocorticoid nuclear receptor (MR), and that the detrimental effects can largely be blocked by spironolactone, an antagonist of MR. As yet, it is not known if these effects are common to other ecdysteroids, e.g., 20E.

33.2.6.4 Potential for Environmental Endocrine Disruption

As indicated above, the toxicity and safety data for 20E and the few other ecdysteroid analogues which have been in any way assessed do not indicate that dietary or environmental contact would have negative impacts on mammals. However, significant releases of ecdysteroids into the environment, either as a by-product of the isolation of large amounts of 20E for pharmaceutical/nutraceutical purposes or as the consequence of excretion of 20E or its metabolites by those taking doses (probably 0.5–5 g/person/day) for medical, health, or aesthetic reasons, might have serious detrimental effects on arthropods and other invertebrates, with aquatic insects and crustaceans being particularly susceptible if waste water and sewage are not appropriately treated before it enters natural water courses (Lafont and Dinan 2009). It will be necessary to determine the extent to which ecdysteroids are subjected to microbial degradation in passing through a sewage works and whether they can be readily removed from purified water by adsorption onto charcoal or reverse-phase materials. One can also envisage that signal invertebrate species (e.g., Asada et al. 2014) or cell-based bioassays (Dinan 1995a; Pounds et al. 2002) could be used to detect and monitor for the release of ecdysteroid agonists into the environment and thereby avoid serious environmental impacts.

33.2.7 Marketed Products

The Verde Vital series of commercial supplements (www.verdevital.de) contain spinach powder in preparations with other plant extracts, vitamins, minerals, fatty acids, creatine, resveratrol, etc. with designations for osteoporosis, muscle, joint, heart, and circulation problems and for improved stamina. The website indicates that

the daily dose for most of the supplements in the series contains 400–900 mg of "ecdysone," but this seems questionable since E is a minor ecdysteroid in spinach, and it may be that the amounts refer to the mass of spinach powder, in which case the amount of 20E would be ca.0.5–1 mg/day. Additionally, it has been questioned whether the ecdysteroid profile of these products is compatible with spinach alone, and evidence has been provided that ecdysteroids from another plant species (*Cyanotis arachnoidea*) have been added; quantification of 20E revealed 2–24 mg in daily doses of the various preparations (Hunyadi et al. 2016).

33.2.8 Relevant Patents

Over 275 separate patents concerning phytoecdysteroid preparations and their pharmaceutical, medical, and nutraceutical applications have been published over the last 50 years, with the number per annum increasing exponentially over the period. It is not possible to review all these patents in the context of this chapter, so we shall add the list of relevant patents to Ecdybase. Also, it is possible to access most patents on Google Patents, searching by criteria (key terms, year, inventor, etc.), and often the patent can be read in several languages.

33.2.9 Perspectives

Present evidence indicates that ecdysteroids are currently rare, nontoxic components of the human diet, with only those relatively small population groups (Andean Indians etc.) which consume a significant proportion of amaranth crops (quinoa, etc.) potentially receiving significant amounts of ecdysteroids, mainly 20E. It should be determined how much ecdysteroid those for whom quinoa or other amaranths are staple foods really do consume once it has been cooked, whether the pharmacokinetics of ecdysteroids are different in these people through genetic selection or induction of enzymes or other relevant biological processes and whether they are less susceptible to conditions which the consumption of ecdysteroids is believed to counteract (e.g., sarcopenia, type 2 diabetes).

One can expect the Western diet to undergo major changes over the next few decades, driven by two major factors: climate change and the need to reverse the spread of metabolic syndrome. Reduction in the consumption of meat, together with greater proportion and diversity of vegetables in the diet, the medicinal use of ecdysteroids, social health uses as adaptogens, anabolics, etc., can all be expected to increase the intake of phytoecdysteroids. Enhanced levels of ecdysteroid in crop plants could arise from increasing the levels of these compounds by GM or non-GM means for crop protection purposes or for health/nutraceutical reasons (e.g., using the diet to help counteract the effects of aging and/or inactivity).

Clearly, for this to become feasible, we need fundamental data on the elucidation and characterization of the biosynthetic pathway(s) for ecdysteroids in plants and a thorough understanding of how their synthesis and metabolism are regulated. Only then will it be possible to devise rational strategies for enhancing ecdysteroid levels

in other crop species and also for controlling the regulation so that the desired analogues are produced at the desired concentrations, at the right time, and in the correct parts of the plant to optimize crop protection or nutraceutical value.

On the other side of the equation is our currently incomplete understanding of the mode(s) of action, pharmacokinetics, and metabolism of ecdysteroids in mammals and particularly in humans. The low toxicity and plethora of beneficial effects which have been ascribed to ecdysteroids are encouraging for their future development, but the low bioavailability means that ingested doses of 20E have to be relatively large, which increases cost and raises supply problems (especially as 20E can only be realistically obtained by isolation from a rich plant source; 20E is chemically too complicated for efficient commercial chemical synthesis) and the potential release of an invertebrate endocrine disruptor into the environment.

In theory, bioavailability might be increased by improved formulation, modified routes of application (e.g., sublingual or dermal implants; Dittrich et al. 2000), or the use of new analogues with greater potency (improved bioavailability, reduced metabolism and/or rate of excretion, enhanced biological activity), but since 20E is currently the only ecdysteroid which can be isolated economically in adequate amounts and semisynthetic routes from this analogue would have to be high yield, the options are presently restricted.

The mode(s) of action of ecdysteroids in mammalian systems needs to be fully established. It is clear that it is very different from the main modes of action of ecdysteroids in insects or crustaceans and that it involves signalling from the plasma membrane, but the precise nature and whether the same or different signalling systems operate in different mammalian target tissues need to be determined. As for vertebrate steroid hormones, membrane-, as well as nuclear-, receptors for ecdysteroids have relatively recently been detected in invertebrate systems (e.g., Elmogy et al. 2004; Srivastava et al. 2005), and thus research on ecdysteroid membrane receptors in mammals and invertebrates can be expected to cross-fertilize each other.

Only after concerted effort to understand the bioavailability, mode of action, metabolism, and excretion of ecdysteroids in specific mammalian systems will it be possible to fully exploit the extensive range of analogues which already exist to identify more active analogues and more potent analogues which possess better bioavailability or resistance to metabolism and excretion.

The historical and continuing traditional medicinal uses of ecdysteroid-containing plants and the large number of scientific publications attesting to a plethora of essentially beneficial pharmacological effects in mammals, including humans, attest to the great promise and strong potential of ecdysteroids for the development of a new class of drug for the treatment or at least slowing the progress of several currently intractable muscle wasting diseases and modern societal syndromes, such as obesity or type 2 diabetes. The way to reach this goal is long and demanding, as it requires companies to invest much time, effort, and money into the identification of a good plant source of 20E, secure a reliable supply of the compound, develop an economically viable industrial-scale procedure for the preparation of pharmaceutical grade drug; obtain thorough evidence for the effectiveness, efficacy, pharmacokinetics, and mode of action in animals and humans; and successfully complete all the necessary clinical trials. Preclinical and clinical trials of 20E have recently been initiated for a

number of designated disease conditions (sarcopenia, Duchenne muscular dystrophy) and can be expected to be completed within the next year. This is reassuring because although the first data on the effects of ecdysteroids on mammals goes back almost 50 years, the area had been very deficient in thorough clinical trials because enough pure 20E simply was not available.

33.3 Conclusions

Phytoecdysteroids accumulate in a wide range of plant species where they serve to deter invertebrate predation. In this context, ecdysteroids are currently natural components of a limited number of natural food plants, with quinoa and spinach being responsible for the largest amounts of ecdysteroids in the human diet. 20E, at least, is nontoxic and appears to have many positive biological effects in mammalian systems. Thus, one could envisage the amount of dietary ecdysteroids significantly increasing in the near future, if (i) ecdysteroid levels are elevated in other crop species either to enhance their resistance to predation or to take advantage of the promising biological activities of the ecdysteroids, (ii) phytoecdysteroids become established as therapeutic agents for muscle wasting diseases or type 2 diabetes, and (iii) phytoecdysteroid-containing preparations become more popular as anabolic and adaptogenic dietary supplements among specific groups (sportsmen, body builders, etc.) or as tonics for the general public. There remains a strong need to elucidate biosynthesis and regulation of phytoecdysteroid biosynthesis and to clarify the pharmacokinetics and mode of action in responding mammalian cells, but ecdysteroids are showing considerable promise and potential as therapeutic agents of the future.

33.4 Cross-References

► Antioxidants in Diets and Food

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Alkaloids in Diet

34

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Contents

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Abstract

Food is one of the three basic requirements of mankind, supplying six kinds of nutrients including water, carbohydrate, protein, lipid, vitamins, and minerals. Alkaloid-containing foods are an intrinsic part of the human diet, such as tea, coffee, and tomato. These food-oriented alkaloid constituents possess diverse effects on the human body, either wanted or unwanted. A large variety of food-produced alkaloids exhibit potent bioactivities, such as caffeine, atropine, and cocaine, whereas, lots of other alkaloids are toxic to human, such as pyrrolizidine alkaloids. This chapter focuses on the alkaloids in human diet and their mode of action and possible toxic effects. To organize this chapter, the alkaloids were categorized into nine groups based on their structures: pyrrolizidine alkaloids, tropane alkaloids, quinolizidine alkaloids, isoquinoline alkaloids, quinoline alkaloids, glycoalkaloids, purine alkaloids, pyridine alkaloids, and amide alkaloids. The structures of food-derived alkaloids are described, and their pharmacological activities, bioavailability, metabolism, and toxicological effects are discussed. Moreover, the application of alkaloids in medicines and food supplement, patents, as well as a conclusion about their current impact on food safety are reviewed. The main purpose of this chapter is to provide a comprehensive and up-to-date state of knowledge from phytochemical, pharmacological, and toxicological studies performed on alkaloids in human food.

Keywords

Alkaloids · Bioactive constituents · Toxicity · Bioavailability · Safety

34.1 Introduction

Alkaloids are a class of nitrogen-bearing organic compounds, which are produced by a large variety of organisms, including bacteria, fungi, plants, and animals. More than 13,000 alkaloids have been isolated and structurally elucidated. Plants are the major sources of alkaloids, especially certain families of flowering plants, including Papaveraceae (poppy), Amaryllidaceae (amaryllis), Ranunculaceae (buttercups), Solanaceae (nightshades), and Stemonaceae. Commonly, a given species contains only a few alkaloids with the same core structure, such as the opium poppy (*Papaver somniferum*) and the ergot fungus (*Claviceps*) each containing about 30 different morphine and ergoline alkaloids, respectively. Animal-produced alkaloids are seldom reported, such as the New World beaver (*Castor canadensis*) and poison-dart frogs (*Phyllobates*). Interestingly, alkaloids are widely existed in regular human diet, either intrinsic constituents of vegetables and drinks or food contaminants and flavorings from food processing.

The function of alkaloids in plant is largely unknown. Alkaloids were originally considered as the by-products of plants' metabolic processes, such as the biosynthesis of amino acids. More and more evidence has suggested that some alkaloids serve specific physiological function in plants, such as to protect some plants against certain microorganisms, insects, or animals, to mediate signaling transduction in plants, and to stimulate seed formation and ripeness.

Alkaloids exist in plant tissues as water-soluble salts of organic acids and esters or combined with tannins or sugars rather than as free bases. In their pure form, most alkaloids are colorless, nonodorous, nonvolatile, and crystalline solids. The free bases of alkaloids are prone to dissolve in nonpolar organic solvents, such as chloroform and ether. On the contrary, the salts of alkaloids are soluble in water or dilute acids. The differences in the solubility of alkaloids, depending on their forms, are used in laboratory and industry for the extraction and production of pharmaceutically acceptable products. They also tend to have a bitter taste, such as quinine which is used as a bitter principle in tonic water.

The structural diversity of alkaloids is extremely significant. Most alkaloids have one or more of their nitrogen atoms as part of a ring structure. Biosynthetic precursors of most alkaloids are amino acids, including ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, aspartic acid, and anthranilic acid. The biosynthetic pathways of alkaloids are too numerous and mostly remained unknown at the current stage.

Alkaloids possess diverse and significant physiological effects on humans and other animals (both wanted and unwanted); thus they have been used for thousands of years as medicines, poisons, stimulants, insecticides, aphrodisiacs, and narcotics. Morphine is the first alkaloid to be isolated and crystallized in 1804, as the active constituent of the opium poppy, which is a powerful narcotic used for the relief of pain. Codeine, the methyl ether derivative of morphine from the opium poppy, is an excellent analgesic. Ergonovine (from the fungus *Claviceps purpurea*) and ephedrine (from *Ephedra* species) function as blood vessel constrictors. Ergonovine is used to reduce uterine hemorrhage after childbirth, and ephedrine is used to relieve discomfort from common colds, sinusitis, hay fever, and bronchial asthma. Quinidine, widely distributed in plants of the genus *Cinchona*, has been used to treat arrhythmias and irregular rhythms of heartbeat; quinine (from *Cinchona* species) is used to treat malaria. The alkaloid curare (from *Chondrodendron tomentosum*) is used as a muscle relaxant in surgery. Two alkaloids, vincristine and vinblastine (from *Vinca rosea*), are widely used as chemotherapeutic agents in the treatment of many types of cancer.

Alkaloids are often classified on the basis of their chemical structures. To organize this chapter, the common alkaloids in human diet were classified into nine groups based on their core structures: pyrrolizidine alkaloids (PA), tropane alkaloids (TA), quinolizidine alkaloids (QA), isoquinoline alkaloids, quinoline alkaloids, glycoalkaloids (GA), purine alkaloids, pyridine alkaloids, and amide alkaloids. The core structures of the nine types of alkaloids were shown in Fig. 1. For each type of alkaloids, the occurrence in food and their chemical structures are described, their pharmacological activities, bioavailability, metabolism, and the

application in food (including correctly cooking foods rich in phytochemicals) are introduced, and the marketed products, toxicological effects, and existing safety assessments are discussed.

34.2 Pyrrolizidine Alkaloids

PAs, also called as necine bases, are a group of naturally occurring alkaloids with a core structure of pyrrolizidine (Fig. 1). More than 660 PAs and their N-oxides have been identified in around 6,000 plants, and more than half of them exhibit hepatotoxicity. PAs are produced by plants as a defense mechanism against insect and herbivores. They are found frequently in plants from the families Boraginaceae, Asteraceae (Senecioneae and Eupatorieae), Orchidaceae, and Fabaceae (*Crotalaria*), less frequently in the families Convolvulaceae and Poaceae.

Many kinds of foods have been reported to produce PAs, including borage (*Borago officinalis*), comfrey (*Symphytum officinale*), *Gynura bicolor*, and *Emilia sonchifolia*, to possess PA contamination, such as honey. Food-containing PAs are mostly esters of 1-hydroxymethyl-1,2-dehydro-pyrrolizidine, which always bear a hydroxyl group on carbon 7. Some examples of PAs in food were shown in Fig. 2. In grain commodities, the PAs are considered to originate from seeds or plant fragments of PA-containing weeds (ranging from 50 to 6,000 mg/kg). The consumption of PA-contaminated grains causes acute or chronic liver toxicity. Symptoms of acute PA poisoning include abdominal pain, ascites, nausea, vomiting, diarrhea, dropsy, and very rare, jaundice and fever. PA poisoning is associated with hepatic vein occlusive disease (HVOD) involving obstruction of the small veins with sudden hepatomegaly (enlarged liver) and ascites and may end with death (Dharmananda 2004). According to the World Health Organization (WHO), the lowest daily intake of PAs that causes adverse effects in a human is 0.015 mg/kg body weight,

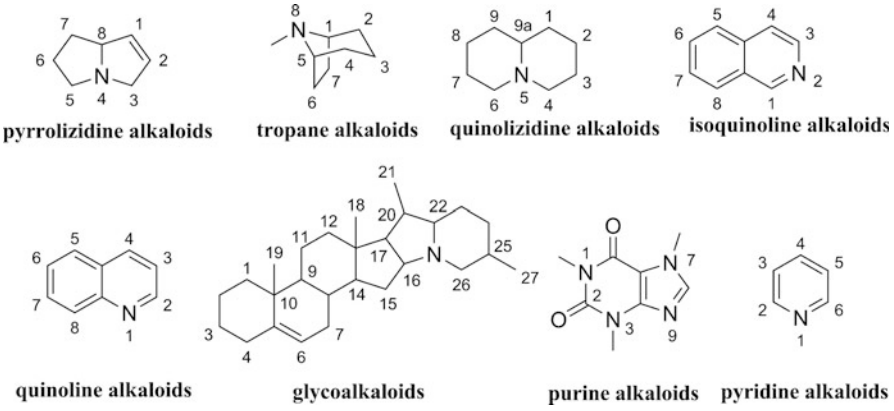


Fig. 1 The core structures of pyrrolizidine alkaloids, tropane alkaloids, quinolizidine alkaloids, isoquinoline alkaloids, quinoline alkaloids, glycoalkaloids, purine alkaloids, and pyridine alkaloids

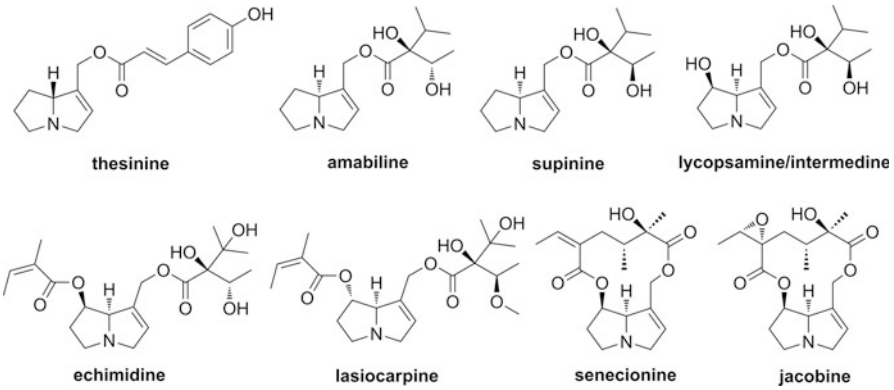


Fig. 2 Some representative PAs in the plant-derived food

corresponding to 0.9 mg/day for a 60 kg person, based on the use of comfrey over a period of 4–6 months (Dharmananda 2004). PAs exert fetotoxic and teratogenic effects at higher doses (Wiedenfeld et al. 2008).

PAs are metabolically activated within the liver, and they can alkylate both proteins and DNA molecules; they are therefore hepatotoxic causing liver damage, as well as mutagenic and carcinogenic. The major metabolic pathways of PAs in human are described as follows: Ester hydrolysis and N-oxidation of PAs represent detoxification processes (Prakash et al. 1999). Toxicity occurs via dehydrogenation of the pyrrolizidine nucleus to generate dehydro-pyrrolizidine moiety (pyrrolic derivatives), followed by acid-catalyzed cleavage of the C7-O bond, resulting in the formation of a carbocation. Then, carbocation may react with available nucleophiles like DNA, leading ultimately to liver necrosis and tumor formation (Prakash et al. 1999).

34.2.1 Borage (*Borago officinalis*)

Borago officinalis (Boraginaceae) is known as starflower, borage, burrage, bourrache, and bugloss. Borage is a plant being widely used in pharmaceutical, industrial, and forage fields and is also being used to make drinks and salads. Borage is native to Europe, North Africa, and Asia Minor and is now being cultivated worldwide. Historical documents showed that borage was firstly cultivated in North Africa and then spread to Spain and other regions.

Borage is a common vegetable in Germany, the Spanish regions of Aragon and Navarre, the Greek island of Crete, and the northern Italian region of Liguria. The plant parts of borage are used in the following ways: leaves made into tea, washes, and poultices; flowers eaten; seeds pressed for oil; and tinctures made from leaves and flowering tops. One of the well-known German borage recipes is the green sauce (grüne soße) made in Frankfurt. In Italian Liguria, borage is commonly used as a filling of the traditional pasta ravioli and pansoti. The flowers or petals of borage are

edible and often used as a garnish on salads and soups and used to flavor pickled gherkins in Poland.

Borage leaves have been used in European herbal medicine since the Middle Ages and are mentioned by Pliny, Dioscorides, and Galen. The name "borage" derives from the Medieval Latin "burra," meaning rough-coated, which refers to the hairs. An alternative explanation suggests it is a corruption of the Latin "corago" (courage). This is in line with its reputation as an herb to dispel melancholy. Borage leaves also have been used to treat rheumatism, cold, and bronchitis, as well as to increase lactation in women. Infusions of the leaves were used to induce sweating and diuresis. Before the invention of ice, borage was used in a cooling drink called a "cool tankard" or "claret cup" consisting of wine, water, lemon, sugar, and borage leaves and flowers. Borage is still widely used in British herbalism, but its use has been waned in North America.

Pliny the Elder believed borage to be antidepressant, and it has long been thought to give courage and comfort to the heart. One old wives' tale states that if a woman slipped a bit of borage into a promising man's drink, it would give him the courage to propose. In European traditional medicine, the heart was believed to store the vital spirit and circulate it around the body via the arteries. Thus "heart medicines" were usually medicines for the spirit – for depression and confusion. Lemon balm (*Melissa officinalis*), lily of the valley (*Convallaria majalis*), motherwort (*Leonurus cardiaca*), and borage are specifics for matters of the heart. These remedies were used to protect the heart from excess heat in high fevers, and borage was much favored for this. Motherwort and borage are a useful combination in thyrotoxicosis, which is a modern version of "excess heat attacks the heart."

The leaves of borage were found to contain a high amount of PAs (10 mg/kg) (Dharmananda 2004). Thesinine (Fig. 2) is one of the few nontoxic PAs produced by plants and is responsible for the deep blue color of the flowers of borage. It tastes sweet, honey-like, and is one of the few blue but edible compounds. Borage also produces small amounts of the poisonous PAs, including amabiline, supinine, intermedine and its enantiomorph lycopsamine, and their 7-acetyl derivatives (Fig. 2). Amabiline and supinine are structural analogues of indicine, a predominant PA in the seeds from plants of the *Heliotropium* species, which might contaminate cereals and grains intended for human and animal consumption. It is not recommended that borage leaves be taken long term internally because of the concentration of PAs that can damage the liver. Some recommend limiting its use to 4–6 weeks, others say 2–3 months at a time. Most sources specify low doses and limited use. Young leaves of borage have been shown to contain less PAs than older ones. Do not take borage if the persons are taking anticoagulants. Borage can cause nausea, cramping, bloating, and headache, although they are relatively mild. Currently, borage is not recommended during pregnancy or lactation, but it has been traditionally used as a galactagogue. Additionally, the hairs on the fresh leaves can irritate the skin.

PAs are also present in borage seeds oil but may be removed by processing. Analysis of borage seed oil showed the possible existence of PAs to induce side effects. Borage seed oil is used for chronic skin inflammatory disease, skin itch, and stimulation problems.

34.2.2 Comfrey (*Symphytum officinale*)

Comfrey (*Symphytum officinale*) is commonly found throughout Europe and parts of Asia and North America, which has been used as a herbal medicine for the treatment of painful muscle and joint complaints for more than 2,000 years. Comfrey is commonly called knitbone because of its amazing ability to heal broken bones and "knit" them back together again. The botanical name, *Symphytum*, means "to unite." Comfrey is used to treat upset stomach, ulcers, heavy menstrual periods, diarrhea, bloody urine, persistent cough, and chest pain as well as is applied to the skin for wounds, joint inflammation, bruises, and rheumatoid arthritis. The leaves and roots of comfrey are also used as herbal teas and vegetable (e.g., in salads).

Several PAs, including echimidine and lasiocarpine (Fig. 2), have been isolated from comfrey. The PA content in comfrey leaves varies from 20 to 1,800 mg/kg. Comfrey root contains PAs with 1,2-unsaturated necine ring structures, almost entirely in the form of their N-oxides, the main ones being 7-acetylintermedine and 7-acetyllycopsamine together with smaller amounts of intermedine and symphytine (Fig. 2). The total amount of PAs given by different authors varies from 0.013% to 1.2% based on the analytical methods.

The major hepatotoxic manifestation in humans ingesting comfrey is hepatic VOD, also called sinusoidal obstruction syndrome (SOS). Several cases of VOD/SOS associated with comfrey ingestion were reported in humans, as well as in experimental animals. Comfrey-induced dose-dependent hepatic VOD was found in rats that were gavaged with a single dose of 200 mg/kg of the mixed PA or 50 and 100 mg/kg thrice a week for 3 weeks. The mechanisms underlying comfrey-induced genotoxicity and carcinogenicity are still not fully understood. The available evidence suggests that the active metabolites of PAs in comfrey interact with DNA in liver endothelial cells and hepatocytes, resulting in DNA damage, mutation induction, and cancer development.

In 2001, the US Food and Drug Administration issued a ban on comfrey products marketed for internal use and a warning label for those intended for external use (FDA/CFSAN 2007). Nowadays, only pyrrolizidine-depleted or pyrrolizidine-free extracts are used in proprietary medicinal products. Comfrey should not be used during pregnancy and lactation, in infants, and in people having liver, kidney, or vascular diseases. To date, the activity-determining constituents and mechanisms of action of comfrey are only partly known. In accordance with the modern approach of evidence-based medicine, comfrey extract creams have been demonstrated their efficacy and tolerability in a number of muscle and joint injuries, such as acute myalgia in the back area, and in blunt injuries. Comfrey herb has also been shown to be efficacious in wound healing. Comfrey root has also been proven to be efficacious in activated osteoarthritis and equivalent or more efficacious in distortions compared with topical diclofenac. It could therefore be promising to investigate topical comfrey preparations in further indications related to muscle or joint pain, for instance, chronic forms of back pain.

34.2.3 Honey

Honey is a sweet, viscous food substance produced by bees and some related insects. Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew). Honey production and use have a long history, depicted in Valencia, Spain, by a cave painting of humans foraging for honey at least 8,000 years ago. Over its history as a food, honey is used as a spread on bread; an addition to various beverages, such as tea; and a sweetener in some commercial beverages. Honey barbecue and honey mustard are other common flavors used in sauces. Honey is also used to make mead beer, called "braggot."

Honey made by bees foraging on *Senecio jacobaea* (tansy ragwort) contain senecionine and jacobine, with the total PA content ranging from 0.3 to 3.9 mg/kg (Fig. 2). The highest level of PAs is 3.9 mg/kg in honey from *Senecio jacobaea*; because this value was not corrected for extraction efficiency, the amount of PAs might be higher.

Plant genera-producing PAs are important contributors to honey production in many countries. Honey made by bees foraging mainly on *Echium* spp. contains echimidine as the major alkaloid (Fig. 2). Analysis of honey available on the German/European market revealed that 19 samples (9%) contained PAs within the 216 samples, in the range of 0.019–0.120 mg/kg, calculated as retronecine equivalents (Kempf et al. 2008).

Levels of PAs present in honey from PA-containing plants are well above that considered by the German Federal Health Bureau. Long-term consumption of PA-containing honey is capable of causing progressive chronic toxicity, especially in infants and fetuses. Although no incidents of PA poisoning have been reported due to consumption of honey, a report issued by the International Programme on Chemical Safety (IPCS) concluded that the level of PAs in honey may contribute to chronic liver disease or liver tumors (Edgar et al. 2002).

34.3 Tropane Alkaloids (TAs)

TAs are a class of bicyclic [3.2.1] alkaloids and secondary metabolites that contain a tropane ring in their chemical structure. TAs are found in plants of numerous families, especially Solanaceae, Erythroxylaceae, Convolvulaceae, Brassicaceae, and Euphorbiaceae, and they comprise mono-, di-, and tri-esters and carboxylated and benzoylated tropanes. More than 200 TAs have been isolated and identified. Several of these alkaloids occur as chiral structures due to the presence of a tropic acid residue attached to the ecgonine nucleus as an ester. The former occurs naturally in its *R* form; however, racemic mixtures may appear, especially during alkaline extraction (e.g., the formation of (+)-atropine from (−)-hyoscyamine). Several acids are distinguished as being present in the TAs, including tropic, tiglic, acetic, isovaleric, isobutyric, benzoic, or anisic acids.

TA-containing plants have been used medicinally and for folkloric purposes in many countries. Atropine, hyoscyamine, and scopolamine are used therapeutically

for different medical indications. TAs are commonly used as anti- colic and spasmolytic drugs (scopolamine) in both digestive and urinary tract spastic conditions. Especially, atropine is commonly used in ophthalmological eyedrops to enlarge pupils, paralyze the accommodation reflex, and enable the ophthalmic examination. The juice from *Atropa belladonna* was extensively used by women in the time of the Renaissance to enlarge the pupils of the eyes so as to improve their looks. The bioactivities of TAs are relied on the antagonistic action on muscarinic acetylcholine receptors (Brown and Taylor 2006). Several TAs are hallucinogenic agents and some are powerful anticholinergic drugs.

Plants synthesize and store TAs as a protection against being eaten (e.g., by insects). Most TAs are toxic to humans. However, TAs are characterized by numerous contraindications and side effects. The effects of TA administration are characterized by dryness of the mucosa of the upper digestive and respiratory tract, constipation, pupil dilatation, disturbance of vision, photophobia, dose-dependent occurrence of hyper- or hypotension, bradycardia, tachycardia, arrhythmias, nervousness, restlessness, irritability, disorientation, ataxia, seizures, and respiratory depression (EFSA 2008b). Oral administration of atropine in single doses of 0.5–1 mg up to three times daily is used to treat smooth muscle spasms in the gastrointestinal tract, which causes side effects including slight cardiac slowing and dryness of mouth (Brown and Taylor 2006). A single oral dose of atropine in the range of 2–5 mg is associated with rapid heart rate, dilated pupils, blurring of vision, difficulties of speaking and swallowing, and dry and hot skin (Meletis and Wagner 2002). Oral doses of 10 mg or more atropine lead to rapid and weak pulse, ataxia, restlessness, excitement, hallucinations, delirium, and coma (Brown and Taylor 2006). Hyoscyamine is used in the treatment of visceral spasm in oral single doses of 0.15–0.3 mg up to four times daily, showing the same adverse effects as atropine (Martindale 2010). Scopolamine is administered orally in single doses of 0.15–0.3 mg up to four times daily in the prevention of postoperative dizziness and motion sickness. Typical adverse effects for anticholinergic drugs such as dryness of the mouth, changes in heart rate, and disturbance of vision are reported within the range of the therapeutically dosage (Martindale 2010). TAs should be avoided in patients with glaucoma, prostatic hypertrophy, and urinary tract diseases and also during pregnancy.

TA intoxications for humans result mainly from abuse (because of the hallucinogenic effects), consumption with TA-containing plants, or accidental exposure (EFSA 2008b). Cocaine is a drug of abuse in many countries. It is the second most popular psychostimulant (after cannabis), temporarily improving mental and physical functions. Cocaine inhibits serotonin, norepinephrine, and dopamine reuptake. In higher doses, cocaine may evoke the blockage of sodium channels resulting in cardiac death. Chronic intake may cause serious transmitter level disorders leading to depressions, suicide attempts, insomnia, or psychomotor retardation. Its abuse resulted in more than 4,000 deaths in 2013. It has local anesthetic properties which are largely forbidden nowadays. After absorption, hydrolysis of TAs inactivates their bioactivities and reduces their toxicity in certain animal species (EFSA 2008b).

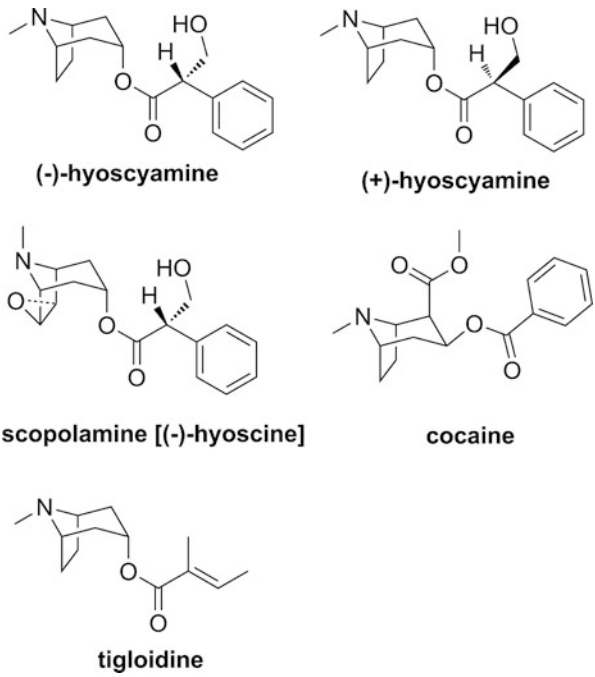


Fig. 3 Structures of some representative TAs in the plant-derived food

The TA-containing foods include wolfberry (*Lycium barbarum* L.), buckwheat (*Fagopyrum esculentum*), soybean (*Glycine max*), flax (*Linum usitatissimum*), Cape gooseberry (*Physalis peruviana*), and coca (*Erythroxylum coca*). The structures of some food-producing TAs were shown in Fig. 3, such as (−)- and (+)-hyoscyamine (a racemic mixture of these two alkaloids is known as atropine), (−)-scopolamine (also known as (−)-hyoscine), and cocaine.

34.3.1 Wolfberry (*Lycium barbarum* L.)

Lycium barbarum L. and *Lycium chinense* Miller (wolfberry, belonging to Solanaceae family) are two closely related species with congruent uses as food and medicinal plants in East Asia. The berries of both species are a very popular ingredient in Chinese cuisine. They are consumed in soups and porridges and added to different meat and vegetable dishes. The young leaves of both species are a valued vegetable.

Wolfberries are not only a vegetable but also a traditional Chinese herbal medicine. Although only *L. barbarum* is officinal in the China Pharmacopoeia, both species, *L. barbarum* and *L. chinense*, have been used medically for more than 2,000 years. In traditional Chinese medicine, wolfberries are used as a mild Yin tonic, enriching Yin in the liver and kidney and moistening lung Yin. Wolfberries are prescribed to treat blurry vision and diminished visual acuity, infertility, abdominal

pain, dry cough, fatigue, and headache. The berries are also praised in the folk medicine to increase longevity and against prematurely gray hair. In a representative study with 42 elderly participants, consumption of 50 mg of wolfberry extract twice a day over 2 months decreased dizziness, fatigue, chest distress, sleep problems, and anorexia. Besides China, wolfberry is also used as herbal drug in other Asian countries, including Vietnam, Korea, and Japan.

Wolfberry is commonly designated as "Himalayan goji berry" or "Tibetan goji berry" on the global functional food market. The variety of commercialized products is considerable. Besides juices, beers, and wines, wolfberry is found in cookies, crispy bars, chocolate, muesli, sausages, and soaps. Wolfberry products are increasingly available in drugstores, "Reformhäuser," and organic food shops. Wolfberries and its related products are legally sold as food or food supplements in USA and Europe. However, these products cannot be promoted as drugs, and therapeutic claims are prohibited.

There have been some controversial reports about the atropine content in the fruits of *L. barbarum*. In 1989, a report showed around 0.95% atropine in the fruits of *L. barbarum* collected in India. In a systematic investigation of wolfberries from various provenances, only trace amount of atropine was detected with high-performance liquid chromatography-mass spectrometry (HPLC-MS) method, maximally 19 ppb (w/w) among the analyzed samples (Adams et al. 2006). The presence of atropine in the roots of wolfberry was reported, which is much higher than that in the fruits. In 2006, the US Food and Drug Administration sent warning letters to two wolfberry juice distributors in violation of marketing their product as a drug intended for the prevention or cure of disease, when wolfberry juice is not generally recognized as safe and effective for various health benefits.

The LD_{50} value of a water extract of wolfberries is 8.32 g/kg by subcutaneous application in mice (Potterat 2010), which confirms the virtual absence of toxicity of wolfberry. Although there is no risk with cultivated plants, some caution is advised with samples of unknown origin.

34.3.2 Buckwheat (*Fagopyrum esculentum*)

Buckwheat (*Fagopyrum esculentum*), also known as common buckwheat, Japanese buckwheat, and silver hull buckwheat, is a plant cultivated for its grain-like seeds and as a cover crop. The crop was originated from China, and nowadays, it is widely cultivated over the world. Buckwheat noodles have been consumed by people from Tibet and Northern China for centuries, as wheat cannot be grown in the mountain regions. Nowadays, buckwheat noodles are very popular in the cuisines of Japan (soba), Korea (naengmyeon, mak-guksu, and memil guksu), buckwheat fresh pasta (pasta di grano saraceno) are commonly consumed in Apulia region of Southern Italy and the Valtellina region of Northern Italy (pizzoccheri), and buckwheat groats are commonly used in Western Asia and Eastern Europe. Dehulled seeds (raw groats) are principally used for human consumption as breakfast cereals or as processed flour for making different bakery products and buckwheat-enhanced

nonbakery products (tea, honey, tarhana, and sprouts). Buckwheat is a gluten-free pseudocereal; these products may be included in gluten-free diets for patients suffering from gluten intolerance.

Buckwheat is an important raw material used for functional food because it exhibits a broad range of bioactivities, such as antidiabetic, hypotension, hypocholesterolemic, and hypoglycemic effects. Buckwheat becomes a dietary source of bioactive compounds, such as nutritionally valuable protein, phenolic compounds, starch and dietary fiber, essential minerals, and trace elements. However, TAs are found in buckwheat and its related matrices at concentrations higher than 100 μg/kg. The major source of TA contamination in buckwheat is stramonium (*Datura stramonium*), which produces high concentration of TAs, including atropine and scopolamine. In most temperate climates, stramonium can easily thrive as weeds in buckwheat fields. Despite an adequate management, postharvest handling, and control, some seeds may go undetected to subsequent stages of the food chain making a certain degree of contamination by these TAs unavoidable. Both *Datura* and *Fagopyrum* species in fact produce, and maturate almost simultaneously, a dehiscent fruit-harboring seeds with similar size and weight. It may be particularly relevant for organic agriculture, as a less strict weed management may allow an increased in-field presence of potentially dangerous plants alongside with crops.

34.3.3 Soybean and Flax

Soybean is a species of legume native to East Asia. Soybean is a significant and cheap source of protein for animal feeds and many packaged meals. Soybean products, such as textured vegetable protein (TVP), are ingredients in many meat and dairy substitutes. Approximately 85% of the world's soybean crop is processed into soybean meal and soybean oil. Tofu, soy milk, and soy sauce are among the top edible commodities made from soybeans.

Flax (*Linum usitatissimum*), also known as common flax or linseed, is a species from the family Linaceae. It is a food and fiber crop cultivated in cooler regions of the world. Flax is grown for its seeds, which can be ground into a meal or linseed oil, a product used as a nutritional supplement.

TA contamination has been found in soybean and flax (animal feed). In surveys conducted in Germany, up to 31.1% of soybean and flax products were contaminated with *Datura* seeds; and 65 of the 66 samples contained scopolamine at levels between 0.1 and 33 mg/kg (Bucher and Meszaros 1989).

34.3.4 Cape Gooseberry (*Physalis peruviana*)

The genus *Physalis*, of the family Solanaceae, includes annual and perennial herbs bearing globular fruits, each enclosed in a bladderlike husk which becomes papery on maturity. Of the more than 70 species, only a very few are of economic value. A

species which bears a superior fruit and has become widely known is the Cape gooseberry (*Physalis peruviana* L.), which is used for sauce, pies, and preserves in mild-temperate climates. Reportedly native to Peru and Chile, where the fruits are casually eaten and occasionally sold in markets but is still not an important crop, it has been widely introduced into cultivation in other tropical, subtropical, and even temperate areas.

In addition to being canned whole and preserved as jam, the Cape gooseberry is made into sauce; used in pies, puddings, chutneys, and ice cream; and eaten fresh in fruit salads and fruit cocktails. Because of the fruit's decorative appearance in its showy husk, it is popular in restaurants as an exotic garnish for desserts. To enhance its food uses, hot air drying improved qualities of dietary fiber content, texture, and appearance. In Colombia, the fruits are stewed with honey and eaten as dessert. The British use the husk as a handle for dipping the fruit in icing.

In Colombia, the leaf decoction is taken as a diuretic and an antiasthmatic. In South Africa, the heated leaves of Cape gooseberry are applied as poultices on inflammations, and the Zulus administer the leaf infusion as an enema to relieve abdominal ailments in children. The aerial parts and roots of Cape gooseberry have been shown to contain tigloidine and other secotropane alkaloids (Fig. 3).

34.3.5 Coca

Coca is the leaves of the four cultivated plants in the family Erythroxylaceae, (*Erythroxylum coca* var. *coca*, *Erythroxylum coca* var. *ipadu*, *Erythroxylum novogranatense* var. *novogranatense*, and *Erythroxylum novogranatense* var. *truxillense*) native to western South America. Coca is grown as a cash crop in Argentina, Bolivia, Colombia, Ecuador, and Peru and even in areas where its cultivation is illegal.

Coca is known for its psychoactive alkaloid, cocaine (Fig. 3). The alkaloid content of coca leaves is relatively low. The native people use it as a stimulant, like coffee, or an energy source. Although only the indigenous populations directly chew coca leaves, the consumption of coca tea (Mate de coca) is common among all sectors of society in the Andean countries and is considered to be beneficial to health, mood, and energy. Coca leaf is packaged into tea bags and sold in most grocery stores in the region. Coca-Cola used coca leaf extract from 1885 to 1903. Extraction of cocaine from coca requires several solvents and a chemical process called acid-base extraction.

Traditional medical uses of coca are foremost as a stimulant to overcome fatigue, hunger, and thirst. It is considered particularly effective against altitude sickness. Coca is also used as an anesthetic and analgesic to alleviate pain from headache, rheumatism, wounds, and sores. The high calcium content in coca explains why people used it for bone fractures. Because coca constricts blood vessels, it's also used to stop bleeding. Indigenous use of coca has also been reported to treat malaria, ulcers, and asthma, to improve digestion, to guard against bowel laxity, and to extend

life span. Modern studies have verified a number of these medical applications (Biondich and Joslin 2016).

The major pharmacologically active ingredient of coca is cocaine, with the amount ranging from 0.3% to 1.5% in fresh leaves. Besides cocaine, the coca leaves contain a number of other alkaloids, including methylecgonine cinnamate, benzoylecgonine, truxilline, hydroxytropacocaine, tropacocaine, ecgonine, cuscohygrine, dihydrocuscohygrine, nicotine, and hygrine. When chewed, coca acts as a mild stimulant to suppress hunger, thirst, pain, and fatigue. Almost all of the coca alkaloids are absorbed within 20 min of nasal application, while it takes 2–12 h after ingestion of the raw coca leaves for alkaloid concentrations to peak. When the raw leaf is consumed in tea, around 59–90% of the coca alkaloids are absorbed. The direct consumption of coca leaves does not induce a physiological or psychological dependence or symptoms typical to substance addiction. Due to its alkaloid content and nonaddictive properties, coca has been suggested as a method to help recovering cocaine addicts to withdraw the drug.

Coca is used in the cosmetics and food industries. A de-cocainized extract of coca leaves is one of the flavoring ingredients in Coca-Cola. Coca tea is produced industrially from coca leaves in South America. Coca leaves are also found in a brand of herbal liqueur called "Agwa de Bolivia" (grown in Bolivia and de-cocainized in Amsterdam) and a natural flavoring ingredient in Red Bull Cola. Coca-Cola is an energy drink which is produced in Bolivia from the coca extract.

A health risk assessment of coca leaf extract-containing soft drink concluded that no health risk is to be expected from consumption of this product because of low cocaine content. The lowest dose of cocaine that can cause adverse effect is 4,800 mg per day for an adult. Assuming a high daily consumption of 1.7 L, the margin of safety (MOS) between the consumed amount of cocaine and the amount upward of which adverse effects may occur is a factor of approximately 7,000. The prohibition of the use of the coca leaf was established by the United Nations in 1961, except for medical or scientific purposes. The coca leaf is listed on Schedule I of the 1961 Single Convention together with cocaine and heroin.

34.4 Quinolizidine Alkaloids (QAs)

QAs (norlupinane, octahydro-2H-quinolizine) are nitrogen-containing heterocyclic compounds. The most common QAs include sparteine and lupanine, α-isolupanine, 13α-hydroxylupanine, and anagyrine (Fig. 4). QAs exist in many species of the genus *Lupinus*, commonly known as lupin or lupine. *Lupinus* is a diverse genus; only four species have been domesticated and are agriculturally significant: *Lupinus angustifolius* (NLL), *Lupinus albus* (white lupin), *Lupinus luteus* (yellow lupin), and *Lupinus mutabilis*. Plants from the genus *Lupinus* have been traditionally used as an animal feed and gained recognition as a healthy food. They contain high amount of protein and fiber and possesses certain beneficial nutraceutical properties.

QAs are nontoxic to the legumes that produce them but toxic and in some cases very toxic to other organisms. The toxicity of alkaloids is considered to be connected

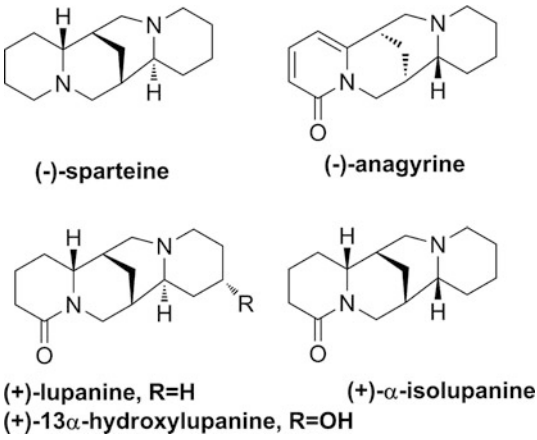


Fig. 4 Structures of some representative QAs in the plant-derived food

with their bitter taste. The QAs are certainly bitter in taste to humans. The most toxic QAs are tetracyclic with a pyridone nucleus. One of these is anagyrine. One case mentions anagyrine being passed into the human body via milk from goats foraging on *Lupinus latifolius*. The anagyrine caused severe bilateral deformities of the distal thoracic limbs in a baby boy. Both sparteine and lupanine display moderate acute toxicity, the former being the more toxic one. The acute oral LD_{50} (lethal dose for 50% of the population) value in rats for the extract of *Lupinus angustifolius* L. is 2,279 mg/kg body weight and for lupanine is 1,464 mg/kg body weight. There are some cases of acute toxicity in humans. According to some results, the LD_{50} value for sparteine is 60 mg/kg, lupanine 159 mg/kg, 13-hydroxylupanine 189 mg/kg, 17-hydroxylupanine 177 mg/kg, and oxolupanine 190 mg/kg. When humans ate lupin beans, which had not been de-bittered, they suffered from blurry vision, dry mouth, facial flushing, and confusion. A young man who drank 0.5 L of water that had been used for the de-bittering of lupin seeds suffered from sudden weakness, palpitations, extra systoles, and different anticholinergic symptoms. In another study, accidental ingestion of unripe lupin seeds resulted in nausea, migraine, abdominal pain, bradycardia, and respiratory depression.

Sparteine is especially present in European *Lupinus* species, whereas lupanine is typical for Australian species. The Australian varieties developed by plant-breeding programs are called "sweet lupins" as they contain a strongly reduced amount of total alkaloids. The mean total alkaloid content of the marketed Australian sweet lupin (*Lupinus angustifolius*) seeds is on average 130–150 mg/kg, of which 70% is lupanine. European lupins, which are predominantly consumed in Southern Europe as seeds (beans), have a high alkaloid content of 10–20 g/kg. The alkaloid level can be reduced through a de-bittering process involving soaking and washing with water. Lupin flour is used to replace a small percentage of wheat flour and soybean flour. It is further used in food formulations to replace soy flour in food commodities and also in lupin-based meals, pastas, pastries, cakes, biscuits, snacks, tempe, bread, miso, soy sauce, dairy/tofu product, and coffee substitutes. For example, a coffee surrogate, made of roasted lupin beans, has an alkaloid content of 200 mg/kg of

product. Lupins can be used to make a variety of foods, both sweet and savory. The European white lupin beans are commonly sold in a salty solution in jars (like olives and pickles) and can be eaten with or without the skin.

QAs are potent antagonists on the nicotinic cholinergic receptor but weak antagonists on the muscarinic cholinergic receptor. Neurological (weakness, dizziness, mydriasis, anxiety, confusion, malaise, loss of coordination, visual disturbances, and dry mouth), cardiovascular (dysrhythmias), and gastrointestinal (nausea, vomiting) symptoms are due to their anticholinergic effects. QAs act via inhibition of ganglionic impulse transmissions of the sympathetic nervous system. It is evident that each QA has its own effect. On the other hand, some QAs are used as folk medicines. They probably have chronic toxicity. However, adequate knowledge about the chronic toxicity of these alkaloids and especially of chronic toxicity across generations is not available. The premise that QAs have not produced hereditary symptoms has not been checked with total reliability.

A risk of lupine allergy exists in patients allergic to peanuts. Indeed, most lupin reactions reported are in people with peanut allergy. Because of the cross-allergenicity of peanut and lupin, the European Commission has required that food labels indicate the presence of "lupin and products thereof" in food.

34.5 Isoquinoline Alkaloids

Isoquinoline alkaloids constitute one of the largest groups of natural substances. These compounds are biogenetically derived from phenylalanine and tyrosine and include an isoquinoline or a tetrahydroisoquinoline ring as a basic structural feature in their skeleton. Isoquinoline alkaloids are widely distributed in plants of the families Papaveraceae, Berberidaceae, Ranunculaceae, Menispermaceae, Fumariaceae, Rutaceae, and Annonaceae. Poppy alkaloids, derived from *Papaver somniferum*, belong to the isoquinoline alkaloids. Isoquinoline alkaloids have a variety of biological activities, including antitumor, antibacterial, analgesic, immune regulation, antiplatelet aggregation, anti-arrhythmia, and antihypertensive effect.

Foods containing isoquinoline alkaloids include opium poppy (*Papaver somniferum*) and lotus (*Nelumbo nucifera*).

34.5.1 Opium Poppy (*Papaver somniferum*)

Papaver somniferum, commonly known as the opium poppy or bread seed poppy, is a species of flowering plant in the family Papaveraceae. In 2016, the worldwide production of poppy seeds was 92,610 tons. The Czech Republic occupied 31% of the total, followed by Turkey and Spain.

Isoquinoline alkaloids occur in the latex of opium poppy. The latex of immature capsules is called opium, which is released by incisions and dried on the capsule surface. Opium contains approximately 20–25% alkaloids. Till now, around 50 isoquinoline alkaloids have been isolated from opium. The main alkaloids of

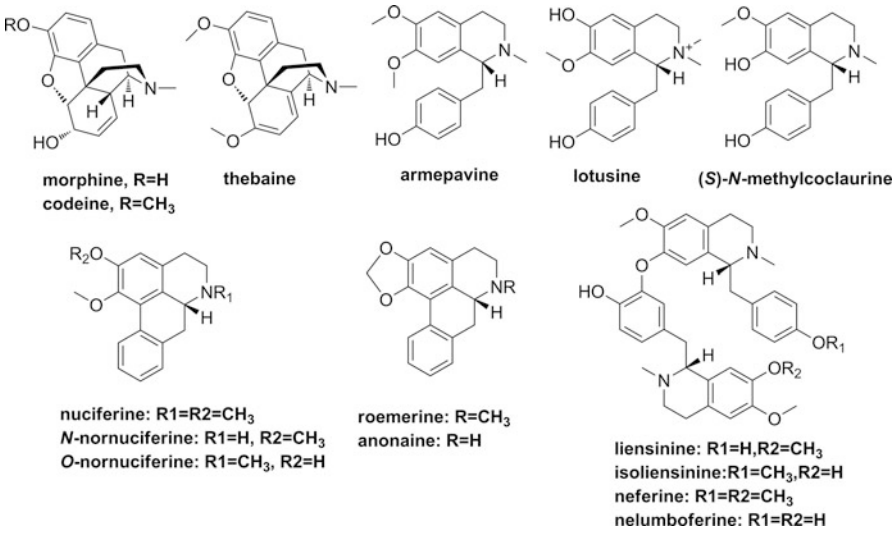


Fig. 5 Structures of some representative isoquinoline alkaloids in the plant-derived food

opium are morphine (depending on origin 7–20%), codeine (0.3–6%), and thebaine (0.2–1%) (Fig. 5).

The seeds of opium poppy are the only part of the plant used as food. The alkaloid content in seeds varies greatly. Morphine (<0.1–620 mg/kg) and codeine (0.1–57 mg/kg) are usually detected in the seeds probably due to contamination with the latex or other parts of the plants. The differences of the alkaloid content in the seeds depend on the geographical origin, soil type, climate, year of harvest, and the cultivar. Washing the seeds prior to use removes as much as 45.6% of free morphine. 10–50% and 98–100% of the morphine content in the seeds can be removed by baking and washing combined with subsequent heating, respectively. The small black, brown, white, or blue seeds are common garnish on bagels, rolls, muffins, and breads in some countries and also used to produce edible oil and jams.

Morphine acts through binding to opioid receptors in the central and peripheral nervous system and the gastrointestinal tract. Activation of the opioid receptors in these organs mediates both the beneficial and the adverse effects of opium alkaloids. Acute morphine intoxication exhibits three symptoms: miosis, respiratory depression, and unconsciousness (coma). Respiratory depression is the most important risk after an opioid overdose. The direct cause of death is respiratory arrest. In individuals who do not show any tolerance development, serious toxic symptoms may occur after the oral administration of 40–60 mg of morphine. For adults, a dose of 200 mg morphine may be acutely lethal. Other studies give a range of 230–1,140 mg of morphine for the oral lethal doses in the case of non-opiate-dependent adults, whereas babies and infants are far more sensitive (Arzneibuch 2004). In animal experiments, morphine had a negative impact on development and reproduction. It has been reported that the consumption of commercially available

poppy seeds, e.g., in the form of desserts with approximately 10–20% poppy seeds or poppy seed cakes, led to light-headedness and enteroparesis in sensitive individuals.

Manufacturers and consumers should be advised to use some methods (e.g., washing, heating, baking) to reduce the contamination of poppy seeds with opium alkaloids. In the case of ingestion of small amounts of poppy seeds used for decoration of pastries, the effects of morphine on human health seem to be negligible.

34.5.2 Lotus (*Nelumbo nucifera*)

Nelumbo nucifera (Nymphaeaceae), which is commonly known as lotus, is a large aquatic herb. The genus *Nelumbo* consists of two species, Asian lotus (*Nelumbo nucifera* Gaertn.), distributed in Asia and the northern parts of Oceania, and American lotus (*Nelumbo lutea* Wild.), distributed in eastern North America. All parts of *Nelumbo nucifera*, including root, leaf, petioles, seeds, lotus core, and lotus house (torus), are edible, with the rhizomes and seeds being the main consumption parts. The rhizomes, leaves, and seeds of *Nelumbo nucifera* have been used as folk medicines, Ayurveda, traditional Chinese medicine, and oriental medicine. The leaves are used for hematemesis, epistaxis, and hematuria; the seeds are used for diarrhea, cholera, fever, and hyperdipsia; and the rhizomes have diuretic, antidiabetic, and anti-inflammatory properties.

The alkaloids are mainly accumulated in lotus leaves and lotus hearts, and their content is as high as 0.6% of their fresh weight (Deng et al. 2016). On the basis of their structures, the alkaloids from lotus can be divided into three categories: monobenzylisoquinolines, aporphines, and bisbenzylisoquinolines. The aporphine-type alkaloids, such as nuciferine, *O*-nornuciferine, *N*-nornuciferine, anonaine, roemerine, dehydronuciferine, and pronuciferine, are mainly distributed in lotus lamina, whereas the bisbenzylisoquinoline-type alkaloids, including liensinine, isoliensinine, and neferineare, predominantly accumulate in the embryo of the seeds (Fig. 5).

These alkaloids have shown a wide range of bioactivities. In the area of neurological disorders, the alkaloids from lotus seeds possess strong inhibitory activities against β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) and butyrylcholinesterase (BChE), suggesting the potential use of *Nelumbo nucifera* in the prevention and treatment of Alzheimer's disease. Further studies showed that the active constituents of *Nelumbo nucifera* that possessed inhibitory effects against BChE and BACE1 are neferine, liensinine, vitexin, quercetin 3-*O*-glucoside, and northalifoline (Je and Lee 2015). Nuciferine has been reported to have a receptor profile similar to aripiprazole-like antipsychotic drugs. Administration of nuciferine blocks head-switch responses and enhances amphetamine-induced locomotor activity, suggesting the potential of nuciferine as antipsychotic drug. Moreover, the lotus leaf alkaloid extract displayed sedative hypnotic and anxiolytic effects via binding to the γ-amino butyric acid (GABA) receptor and activating the monoaminergic system

(Ye et al. 2014). In addition, the total alkaloids from lotus leaf showed sedative hypnotic effect by increasing the brain level of GABA (Yan et al. 2015). Liensinine, isoliensinine, and neferine, isolated from the embryo of *Nelumbo nucifera* seeds, elicit antidepressant-like effects in mice after the forced swimming test.

The alkaloid-rich fraction and neferine possess the potent effect on inhibition of vascular smooth muscle cell (VSMC) proliferation and migration. In addition, neferine and liensinine are effective in preventing the onset of reentrant ventricular tachyarrhythmia. Neferine inhibits angiotensin II-stimulated VSMC proliferation through induction of heme oxygenase-1 and downregulation of fractalkine gene expression (Zheng et al. 2014). Nuciferine increases the phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser(1177), thereby increasing the cytosolic NO level to promote vasorelaxation in human umbilical vein endothelial cells (HUVECs). Under endothelium-free conditions, nuciferine attenuates calcium-induced contraction, suggesting that nuciferine may have a therapeutic effect on vascular diseases associated with aberrant vasoconstriction (Wang et al. 2015b). In addition, the treatment of the *Nelumbo nucifera* extracts significantly suppresses VEGF-induced angiogenesis (Lee et al. 2015). Furthermore, the hydroalcoholic extract of *Nelumbo nucifera* seeds showed antiestrogenic effect without altering the general physiology of reproductive system.

The potential benefit of (*S*)-armepavine on systemic lupus erythematosus was studied on MRL/MpJ-lpr/lpr mice, a model to study human systemic lupus erythematosus. (+)-(*R*)-coclaurine and (−)-(*S*)-norcoclaurine, quercetin 3-*O*-β-D-glucoside, aporphine, liensinine, neferine, and isoliensinine from *Nelumbo nucifera* leaves possess potent anti-HIV activity. Nuciferine decreases hyperuricemia in potassium oxonate-induced rats (Wang et al. 2015a).

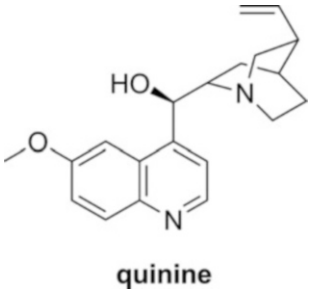
Nuciferine inhibits nicotine-induced non-small cell lung cancer via inhibiting proliferative Wnt/B-catenin signaling pathway and its downstream targets including c-myc, cyclin D, and VEGFA (Liu et al. 2015). Similarly, three major bisbenzylisoquinoline alkaloids, isoliensinine, liensinine, and neferine, show anti-cancer activity in triple-negative breast cancer cells (Zhang et al. 2015).

34.6 Quinoline Alkaloids

The best-known quinoline alkaloid is quinine (Fig. 6). Quinine occurs in considerable amounts in the bark of *Cinchona* species, e.g., *C. pubescens* and *C. officinalis*, from which it is isolated for pharmaceutical and food uses. It is applied to treat malaria (650 mg of quinine sulfate every 8 h for 3–7 days) and nocturnal leg cramps (200–400 mg of quinine sulfate dihydrate/day for a maximum of 5 weeks). *Cinchona* is a genus of flowering plants in the family Rubiaceae, containing at least 23 species of trees and shrubs. *Cinchona* is the only economically practical source of quinine, a drug that is still recommended for the treatment of falciparum malaria.

The main dietary exposure to quinoline alkaloids is through beverages. Quinine is a flavor component of tonic water, bitter lemon, and vermouth because of its pleasant bitter taste. In some countries like USA and Germany, nonmedical use of quinine is

Fig. 6 Structure of quinine



regulated. The latest version of the German Flavorings Ordinance of May 2, 2006 (Annexes 4 and 5), gives the following maximum levels in drinks, calculated as quinine: total 300 mg/kg in spirits and 85 mg/kg in nonalcoholic beverages. In USA, the quantity in soft drinks is limited to 83 mg/L (Ballestero et al. 2005).

Quinine exerts physiological effects on skeletal muscles, which has clinical implications. Quinine increases the tension response to a single maximal stimulus delivered to the muscle directly or through the nerves, but it also increases the refractory period of the muscle. The excitability of the motor endplate region decreases so that responses to repetitive nerve stimulation and to acetylcholine are reduced. It may produce alarming respiratory distress and dysphagia in patients with myasthenia gravis. Because of easy placental accessibility, oxytocic action, and embryotoxicity at high doses, pregnancy is a contraindication as are hypersensitivity to *Cinchona* alkaloids, bradycardia, and other cardiac dysrhythmias of clinical relevance, tinnitus, prior damage to the optic nerve, glucose-6-phosphate-dehydrogenase deficiency (symptom: hemolytic anemia), and myasthenia gravis. Quinine may enhance the effects of cardiac glycosides, muscle relaxants, and anticoagulants.

Based on the data available and for the purposes of health protection, the Bundesinstitut für Risikobewertung (BfR) advises against consuming quinine-containing beverages during pregnancy (EFSA 2005). BfR also recommends that people who have existing contraindications should also refrain from consuming quinine-containing drinks. Patients having cardiac arrhythmia and people who take medications that interact with quinine should only drink quinine-containing drinks after consulting their doctors. This applies in particular to medications that inhibit blood coagulation (Dusemund et al. 2010). In view of warnings having been issued about persons who may be particularly sensitive to quinine, the European Food Safety Authority (EFSA) recommends that the toxicological database on quinine should be reconsidered (EFSA 2008a).

34.7 Glycoalkaloids (GAs)

GAs are a group of nitrogen-containing compounds that are naturally produced in various cultivated and ornamental plant species of the Solanaceae family. This large family of plants includes commonly consumed vegetables such as potatoes,

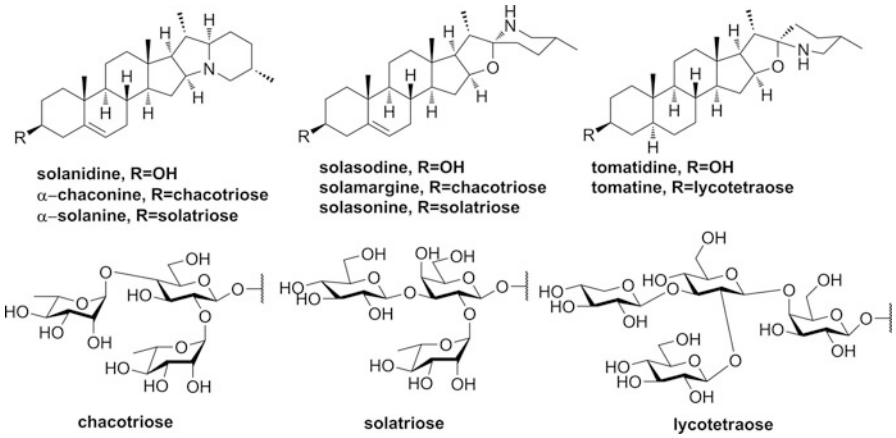


Fig. 7 Structures of GAs in the plant-derived food

tomatoes, eggplants, and peppers. A GA consists of a C27-steroidal alkaloid (aglycone) and a sugar moiety, usually a tri- or tetrasaccharide attached to the 3-OH position. Synonyms for GAs are solanins, *Solanum* alkaloids, steroidal GAs, or basic saponins. Some aglycones, such as solanidine, tomatidine, and solasodine, can form GAs, such as α-solanine and α-chaconine (from potatoes), tomatine (from tomatoes), and solasonine (from eggplant), respectively, when combined with the corresponding glycosides (Fig. 7).

GAs are hardly affected by food processing (baking, cooking, frying), but their content is influenced by the type of cultivar, original total GA content, light, mechanical injury, and storage. GAs (α-solanine, α-chaconine, tomatine, and solasonine) are less toxic than non-glycosylated alkaloids, because the sugar moiety hampers absorption, thereby reducing bioavailability. In vivo hydrolysis results in the formation of aglycones, solanidine, tomatidine, and solasodine, which occurs either by hydrochloric acid in the stomach or bacterial glycosidases in the gastrointestinal tract (Friedman et al. 2003).

34.7.1 Potatoes

Potato is a starchy, tuberous crop from the perennial nightshade *Solanum tuberosum*. Today potato is a staple food in many parts of the world. Potatoes are the world's fourth largest food crop after maize (corn), wheat, and rice. The vegetative and fruiting parts of the potato contain the toxic GAs, including α-solanine and α-chaconine, and are not fit for human consumption. Normal potato tubers that have been grown and stored properly produce small amount of GAs, which are negligible to human health. If the sprouts and skins are exposed to light, the tuber can accumulate a high amount of GAs that can affect human health. Additionally, physical damage and age increase GA content within the tubers.

α-Solanine and α-chaconine poisoning results in gastrointestinal disturbances when consumed at low doses, e.g., vomiting, diarrhea, and usually severe abdominal pain. When consumed at high doses of α-solanine and α-chaconine, neurological disorders occur, including drowsiness, apathy, confusion, weakness, and visual disturbances. The other adverse effects include fever, rapid and weak pulse, low blood pressure, and rapid respiration. GAs from potato cause liver damage, destroy cell membranes, harm the digestive system, and affect metabolism. Breeders try to keep GA levels below 200 mg/kg (200 ppm). McMillan and Thompson reported an epidemic of potato poisoning among 78 school boys after having eaten a lunch with prepared potatoes containing 250–300 mg/kg GAs.

The potato GAs adversely affect intestinal permeability and aggravate inflammatory bowel disease. They are potent irritants of the intestinal mucosa (lytic effect) and cholinesterase (acetyl- and butyrylcholinesterase) inhibitors (Korpan et al. 2004). Based on mice studies, potatoes containing more than 200 mg/kg GAs are considered to cause acute poisoning, but little is known about long-term effects of repeated ingestion of small amounts of potato GAs. This value of 200 mg/kg GAs being of concern with respect to acute poisoning is somewhat different. The results from a limited study in humans cited by Joint FAO (Food and Agriculture Organization)/WHO Expert Committee on Food Additives (JECFA) indicated that the daily consumption of 200–300 g potato tubers containing approximately 240 mg/kg GAs, giving a daily intake of approximately 1 mg/kg body weight/day, did not result in any signs of acute toxicity in humans (JECFA 1993). JECFA also refers to another study where intake of 1–1.5 kg cooked potatoes containing 240 mg/kg GAs, equivalent to about 3.4–5.1 mg/kg body weight, resulted in typical "solanine" poisoning (JECFA 1993).

Control measures should be maintained, especially for potatoes that have been exposed to light and mechanical injury and started to green, which may accumulate 1,000 mg/kg or more GAs. Other applications of potatoes in food such as fried potato skin, potato chips, and baked jacked potatoes may result in increased GA levels than that in boiled potatoes.

34.7.2 Tomatoes

Tomato is the edible, red berry of the nightshade *Solanum lycopersicum*. The species is originated from Western South America. Tomato is consumed in diverse ways, raw in salads or in slices, stewed, incorporated into a wide variety of dishes or sauces, or processed into ketchup or tomato soup. Unripe green tomatoes can also be breaded and fried and used to make salsa or pickled. Tomato juice is sold as a drink and is used in cocktails. Tomatoes are botanically classified as berries, which are commonly used as a vegetable ingredient or side dish.

Tomato contains α-tomatine and a small amount of α-solanine, a toxic alkaloid found in potato leaves and other plants in the nightshade family (Fig. 7). Thus, the application of tomato leaves in herbal tea has been responsible for at least one death. Immature green tomatoes contain up to 500 mg of α-tomatine/kg of fresh fruit

weight. The compound is largely degraded as the tomato ripens until, at maturity, it reaches levels in red tomatoes of around 5 mg/kg of fresh fruit weight. α-Tomatine is a biologically active molecule, as shown by its ability to disrupt cell membranes, bind cholesterol, inhibit acetylcholinesterase, and perturb acid-base equilibria in vivo.

GAs have evolved in nature to protect tomatoes against bacteria, fungi, insects, and animals. It is striking that both green tomatoes and tomato leaves have a very high GA content, which makes them undesirable to eat because the green fruit and leaves not only taste bitter to animals but may not be safe to phytopathogens. Observations that the absence of a 5,6-double bond in the B-ring of tomatidine results in a much less toxic molecule in both pregnant and nonpregnant mice as compared to the structurally similar solasodine (which contains such a double bond) confirm related findings that GAs without such a double bond such as tomatine are less toxic than those which have them such as the potato GAs α-chaconine and α-solanine. It may benefit food safety to create, through plant breeding and/or plant molecular biology methods, plant foods with modified GAs that lack the double bond.

34.7.3 Eggplants

Eggplants (*Solanum melongena* L.), known as aubergine, brinjal, berenjena, or Guinea, is an agronomically and economically important non-tuberous species of the nightshade Solanaceae family. Eggplant has been cultivated for centuries in Asia, Africa, Europe, and the Near East. The fruits of the eggplant are one of the most widely consumed vegetables in the world; however, it is not advisable to eat it raw. In Chinese cuisine, eggplants are often deep-fried. Eggplant supplies low contents of macronutrients and micronutrients. The capability of the fruit to absorb oils and flavors into its flesh through cooking is well-known in the culinary arts.

Eggplants contain several classes of bioactive compounds, including anthocyanidins, flavonoids, saponins, and GAs. Individual or combinations of the bioactive compounds are probably responsible for the reported analgesic, antianaphylactic, anti-inflammatory, antioxidant, antipyretic, intraocular pressure reducing, central nervous system depressing, hypolipidemic, and hypotensive properties of eggplants. Two main GAs found in eggplant are α-solamargine and α-solasonine (Fig. 7). It is well-known that GAs are effective inhibitors of cancer cells due to their toxic effects. Using liquid chromatography-mass spectrometry methods, the contents of the major eggplant GAs (solamargine and solasonine) of *S. melongena* to the allied accession of the Africa-cultivated *Solanum aethiopicum* and *Solanum macrocarpon* were compared. The results show that (a) fruits of *Solanum aethiopicum* and *Solanum melongena* contained (in mg/100 g fresh weight) 0.58–4.56 α-solamargine and 0.17–1.0 α-solasonine and (b) the corresponding much higher values for *S. macrocarpon* are 124–197 and 16–23, respectively, suggesting that this accession might not be safe for consumption. A related study of ten eggplant lines and three allied species (*Solanum aethiopicum*, *Solanum integrifolium*, and

Solanum sodomaeum) confirmed that the allied species had higher GA content than the widely consumed eggplants and that the GA content generally increased during fruit development and ripening. A British study used colorimetry to determine the average GA content of two eggplant samples of about 8 mg/100 g fresh weight. For the adults, 400 mg α-solasonine would be life-threatening. Vegetables in the nightshade family contain a variety amount of solanine, from 2 to 13 mg, and eggplants are found to contain up to 11 mg solanine. Grilling or boiling of eggplants did not result in significant changes in the total GA (solamargine plus solasonine) content.

34.8 Purine Alkaloids

Purine is a heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an imidazole ring. They are the most widely occurring nitrogen-containing heterocycles in nature. Purines are found in high concentration in meat and meat products, especially internal organs such as the liver and kidney. In general, plant-based diets are low in purines. The food-containing purine alkaloids include caffeine, theobromine, 7-methylxanthine, and theophylline (Fig. 8). Purine alkaloids are bitter-tasting alkaloids found in coffee, tea, kola nuts, and cocoa beans. Purine alkaloids, in particular caffeine, exert various effects on metabolic targets (e.g., satiety, thermogenesis, and fat oxidation). The thermogenesis involves inhibiting the phosphodiesterase-induced intracellular degradation of cyclic adenosine monophosphate and antagonizing adenosine receptors that have a negative effect on increased noradrenaline release.

Caffeine is the most consumed psychoactive substance worldwide. It can cause behavior changes in adults (mood, sleep pattern). Caffeine consumption at doses of 50–200 mg/day causes a reduction in drowsiness and fatigue. Consumption of over 200 mg/day caffeine can provoke headache; nervousness; irritability; tremors; central convulsions; negative effects on premenstrual syndrome, fertility, and pregnancy; and cardiovascular effects. Above 400 mg/day caffeine may cause general toxicity like tremor; gastrointestinal problems; cardiovascular problems, including cardiac arrhythmias; and high blood pressure. Oral doses between 5 and 10 g of caffeine are lethal to man (Arzneibuch 2007). Studies on the overall health risks of coffee or caffeine suggested the probability of cancer, including pancreatic,

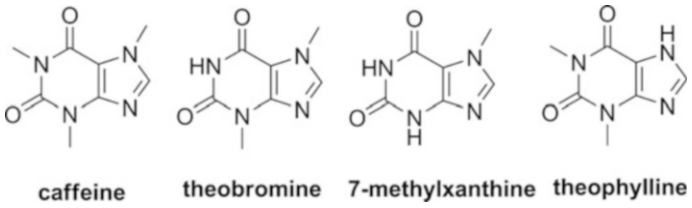


Fig. 8 Structures of purine alkaloids in the plant-derived food

bladder, stomach and ovarian cancers, or leukemia, fibrocystic breast disease, and gallbladder disease (Kotsopoulos et al. 2009). However, no risks of coffee, including decaffeinated coffee and tea for colorectal cancer, were established in epidemiological studies (Michels et al. 2005). Patients with a nonfatal first myocardial infarction taking at least five cups/day have been reported to be prone to increase the risk of a second incidence.

In contrast to the other methylxanthines, the action of theobromine on the central nervous system is weak. The only report about adverse effects showed intake of high doses of theobromine causes nausea and anorexia in humans.

Theophylline is medically used to treat asthma due to its bronchodilating effect. Theophylline toxicity symptoms range from seizures, tachycardia, nausea and vomiting, hypokalemia, headache, and tremors. Cardiac arrest and arrhythmias and hypotension were also frequently recorded in patients or healthy subjects taking theophylline.

34.8.1 Coffee

Coffee is a brewed drink prepared from roasted coffee beans, the seeds of berries from certain *Coffea* species (Rubiaceae). The genus *Coffea* is native to tropical Africa (specifically having its origin in Ethiopia and Sudan), Madagascar, the Comoros, Mauritius, and Réunion in the Indian Ocean. *Coffea* plants are now cultivated in over 70 countries, primarily in the equatorial regions of the Americas, Southeast Asia, Indian subcontinent, and Africa. The two most economically important varieties of *Coffea* plant are the Arabica and the Robusta, which occupy 60% and 40% of the coffee produced worldwide, respectively. In 2016, the worldwide production of green coffee beans was 9.2 million tons, around 33% from Brazil.

Coffee is darkly colored, bitter, and slightly acidic and has a stimulating effect in humans, primarily due to caffeine. Coffee is one of the most popular drinks in the world, and it can be prepared and presented in a variety of ways. Coffee seeds contain 10–20 g/kg caffeine and trace amount of theophylline and theobromine. Brewed coffee and instant coffee contain 360 mg/L and 118–316 mg/L caffeine, respectively, while decaffeinated coffee contains less than 20 mg/L caffeine. A review of clinical trials found that drinking coffee at doses of 3 or 4 cups daily is generally safe and is more likely to improve health outcomes than to cause harm. Exceptions include possible increased risk in women having bone fractures and a possible increased risk in pregnant women of fetal loss or decreased birth weight. Meta-analyses have consistently found that long-term coffee consumption is associated with a lower risk of Parkinson's disease. In a systematic review and meta-analysis of 28 prospective observational studies, representing over one million participants, every additional cup of caffeinated and decaffeinated coffee consumed in a day was associated, respectively, with a 9% and 6% lower risk of type 2 diabetes.

In a healthy liver, caffeine is mostly broken down by the hepatic microsomal enzymatic system. The excreted metabolites are mostly paraxanthines, theobromine

and theophylline, and a small amount of unchanged caffeine. Therefore, the metabolism of caffeine depends on the state of the enzymatic system in the liver.

In 2012, the National Institutes of Health–America Association of Retired Persons (AARP) Diet and Health Study analyzed the relationship between coffee drinking and mortality. They found that higher coffee consumption was associated with lower risk of death and that those who drank coffee lived longer than those who did not. However, the authors noted, "whether this was a causal or associational finding cannot be determined from our data." A meta-analysis found that coffee consumption (4 cups/day) was inversely associated with all-cause mortality (a 16% lower risk), as well as cardiovascular disease mortality specifically (a 21% lower risk from drinking 3 cups/day), but not with cancer mortality (Crippa et al. 2014). Additional meta-analysis studies showed that higher coffee consumption (2–4 cups per day) was associated with a reduced risk of death by all disease causes.

34.8.2 Tea

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of *Camellia sinensis* (the family Theaceae), an evergreen shrub (bush) native to East Asia. Tea is the most widely consumed drink in the world. There are many different types of tea. Darjeeling and Chinese greens have a cooling, slightly bitter, and astringent flavor, while others have vastly different profiles that include sweet, nutty, floral, or grassy notes. Tea originated in Southwest China, where it was used as a medicinal drink. It was popularized as a recreational drink during the Chinese Tang dynasty, and tea drinking spread to other East Asian countries. Tea was introduced to Europe during the sixteenth century.

Caffeine occupies about 1–3% of tea's dry weight, equal to 30–90 mg per 8-oz (250 ml) cup depending on different type, brand, and brewing method. A study found that the caffeine content ranges from 22 to 28 mg in 1 g black tea, while ranged from 11 to 20 mg in 1 g green tea, reflecting a significant difference. Tea also contains small amounts of theobromine and theophylline, which are xanthines similar to caffeine.

It has been suggested that green and black tea can protect against cancer or other diseases such as obesity and Alzheimer's disease, but the compounds found in green tea have not been conclusively demonstrated to have any effect on human diseases. One clinical study demonstrated that regular consumption of black tea over 4 weeks had no beneficial effect in lowering blood cholesterol levels in human.

34.8.3 Kola Nuts

The kola nut is a caffeine-containing nut of evergreen trees of the genus *Cola*, primarily of the species *Cola acuminata* and *Cola nitida*. In folk medicine, kola nuts are used for aiding digestion when ground and mixed with honey and are used for anti-coughs. Kola nuts are perhaps the best known to Western culture as a flavoring

ingredient and one of the sources of caffeine in cola and other similarly flavored beverages, although the use of kola (or kola flavoring) in commercial cola drinks has become uncommon. The kola nut contains up to 3% (30 g/kg) of caffeine, partly bound to tannins. Caffeine-containing drinks including cola drinks and energy drinks contain normally 65–250 mg/L caffeine, which is added to some soft drinks as a flavoring ingredient and to increase the basal metabolic rate.

34.8.4 Cocoa Beans

The cocoa bean, which is also called the cacao bean, is the dried and fully fermented seeds of *Theobroma cacao*, from which cocoa solids (a mixture of nonfat substances) and cocoa butter (the fat) can be extracted. Cocoa beans are the basis of the sweet food preparation chocolate and of the Mesoamerican foods tejate and a pre-Hispanic drink maize.

Cocoa bean contains 35–50% (350–500 g/kg) oil (cocoa butter or theobroma oil), 1–4% (10–40 g/kg) theobromine, and 0.2–0.5% (2–5 g/kg) caffeine, plus tannins and volatile oils. Theobromine is the major alkaloid found in cocoa plants. Due to fermentation and processing, theobromine is hardly found in different cocoa products. Chocolate/cocoa drinks contain 21 mg/L caffeine.

34.9 Pyridine Alkaloids

Pyridine alkaloids are a class of pyridine ring-containing compounds widely found in plants. Foods containing pyridine alkaloids include pepper (*Piper nigrum*), kava (*Piper methysticum*), areca nuts, and *Nicotiana tabacum*.

34.9.1 *Piper nigrum*

Piper nigrum is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning, known as a peppercorn. Peppercorns are described simply as pepper or more precisely as black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit), and white pepper (ripe fruit seeds). Dried ground pepper has been used both for its flavor and as a traditional medicine. Its spiciness is due to the presence of piperine, which is different from the capsaicin characteristic of chili peppers. It is ubiquitous in the modern world as a seasoning and is often paired with salt.

Piperine (Fig. 9) is responsible for the biting, pungent taste of peppers and is used as a spice. The main human dietary exposure comes from this source. Another possible source is brandy to which pepper is added to impart a pungent taste. Black pepper (*Piper nigrum*) contains 5–9% piperine. A daily consumption of 0.33 g of black pepper by a 60 kg person is equal to an intake of 16.5–29.7 mg piperine/person/day.

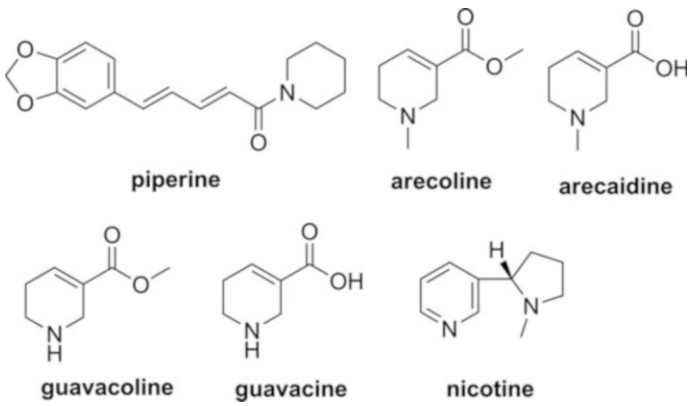


Fig. 9 Structures of pyridine alkaloids in the plant-derived foods

Chronic consumption of black pepper might cause gastric mucosal injury. In a double-blind clinical trial, intragastric administration of the spice (1.5 g) to healthy human volunteers caused severe bleeding in one subject after intake of black pepper. Several cases of fatal pepper administration have been reported in the literature. Mechanical obstruction and mucosal edema were identified as mechanisms of death. However, the observed effects cannot clearly be linked to piperine since piperine was not administered as such. Fatalities/severe side effects might have been caused by mechanical means because exposure was via (accidental) aspiration. Oral intake of large amounts of black and white peppers is regarded as likely unsafe for children and pregnant women. There are insufficient data on possible abortifacient effects in large amounts (Jellin and Gregory 2007). Piperine was found to inhibit human cytochrome P450 3A4(CYP3A4) and P-glycoprotein in vitro, the proteins that are important for the metabolism and transport of xenobiotics and their metabolites (Bhardwaj et al. 2002). By inhibiting drug metabolism, piperine may increase the bioavailability of other compounds (Reen et al. 1993).

Consuming a lower amount of piperine-containing white and black peppers does not represent risks for human health. However, when consumed at higher doses, these peppers might induce unfavorable effects including gastric mucosal injury, and extreme consumers of black pepper may be at increased risk. Due to its effects on drug metabolism, piperine-containing spices should be taken cautiously by individuals taking other medications.

34.9.2 Areca Nuts

The areca nut is the fruit of the areca palm (*Areca catechu*), which grows in the tropical Pacific (Melanesia and Micronesia), Southeast and South Asia, and parts of East Africa. In parts of India, Sri Lanka, and southern China, areca nuts are not only chewed along with betel leaf but also used in the preparation of Ayurvedic and

traditional Chinese medicines. Powdered areca nut is used as a constituent in some dentifrices (Bhat et al. 2013). Other traditional uses include the removal of tapeworms and other intestinal parasites by swallowing a few teaspoons of powdered areca nut, by drinking as a decoction, or by taking tablets containing the extracted alkaloids (Bhat et al. 2013).

Arecoline (methyl-1,2,5,6-tetrahydro-1-methyl-nicotinate) (Fig. 9) is an alkaloid isolated from *A. catechu*, which is considered as the major effective constituent of *A. catechu*. Additionally, arecoline has a wide spectrum of pharmacological activities including effects on nervous, cardiovascular, digestive, and endocrine systems.

For chewing areca nut, a few slices of the nut are wrapped in a betel leaf along with calcium hydroxide (slaked lime) and may include clove, cardamom, catechu (kattha), or other spices for extra flavoring. Arecoline is used in medical treatment for glaucoma. Habitual chewers of betel leaf and areca nut have a greatly increased risk of developing a range of serious diseases, including cancers of the mouth and esophagus (Jain et al. 2017). Chewing areca nut alone has been linked to oral submucous fibrosis. According to MedlinePlus, "Long-term use of betel-areca preparations has been associated with oral submucosal fibrosis, pre-cancerous oral lesions and squamous cell carcinoma. Acute effects of betel chewing include asthma exacerbation, hypertension, and tachycardia. There may additionally be a higher risk of cancers of the liver, mouth, esophagus, stomach, prostate, cervix, and lung with regular betel use. Other effects can include altered blood sugar levels, which may in turn increase the risk of developing type 2 diabetes" (Peng et al. 2015).

34.9.3 *Nicotiana tabacum*

Nicotiana tabacum is an annually grown herbaceous plant. Actually, all plants in the genus *Nicotiana* have been widely cultivated. The leaves are commercially grown in many countries to be processed into tobacco. In their first voyage to the New World, Christopher Columbus and his expedition were introduced to a plant whose smoke was called tobacco by the natives of Hispaniola. In 1560, Jean Nicot de Villemain, the Ambassador of France in Portugal, brought tobacco seeds and leaves as a "wonder drug" to the French court. In 1586, the botanist Jacques Dalechamps gave the plant the name Herba nicotiana, which was also adopted by Linné. It was considered as decorative plant at first, then a panacea, before it became a common snuff and tobacco plant. Tobacco arrived in Africa at the beginning of the seventeenth century. The leaf extract was a popular pest control method up to the beginning of the twentieth century. In 1851, the Belgian chemist Jean Stas documented the use of tobacco extract as a murder poison. The Belgian count Hippolyte Visart de Bocarmé had poisoned his brother-in-law with tobacco leaf extract in order to acquire some urgently needed money. This was the first exact proof of alkaloids in forensic medicine (Wennig 2009).

Pyridine alkaloids are present in tobacco as free bases and salts. Nicotine (Fig. 9) accounts for 90–95% of the tobacco pyridine alkaloids, and nornicotine and anatabine account for roughly 2.5% each. Other pyridine alkaloids are present in

trace amounts including anabasine, myosmine, cotinine, and 2,3′-bipyridyl. *S*(−)-Nn icotine, a major alkaloid in tobacco, is accepted generally as being responsible for maintaining smoking behavior. The reinforcing properties of *S*(−)-nicotine have been demonstrated in laboratory animals using the intravenous self-administration paradigm.

Nicotine's mood-altering effects vary in different reports, both a stimulant and a relaxant. Nicotine stimulates a release of glucose from the liver and epinephrine (adrenaline) from the adrenal medulla, which, in turn, causes stimulation. Users report feelings of relaxation, sharpness, calmness, and alertness (Benowitz 1988). When a cigarette is smoked, nicotine-rich blood passes from the lungs to the brain within 7 s and immediately stimulates nicotinic acetylcholine receptors (Vivekanandarajah et al. 2019); this indirectly promotes the release of many chemical messengers such as acetylcholine, norepinephrine, epinephrine, arginine vasopressin, serotonin, dopamine, and beta-endorphin in parts of the brain (Vivekanandarajah et al. 2019; Pomerleau and Pomerleau 1984). Nicotine also increases the sensitivity of the brain's reward system to rewarding stimuli. At very high doses, nicotine dampens neuronal activity (Kenny and Markou 2006).

A protein of the white-brown complex subfamily can be extracted from the tobacco leaves. It is an odorless, tasteless white powder and can be added to cereal grains, vegetables, soft drinks, and other foods. It can be whipped like egg whites, liquefied, or gelled and can take on the flavor and texture of a variety of foods. It contains 99.5% protein, no salt, fat, or cholesterol. It is currently being tested as a low calorie substitute for mayonnaise and whipped cream.

Nicotine is highly addictive. It is one of the most commonly abused drugs. Generally, a cigarette yields about 2 mg absorbed nicotine. High amounts of nicotine (30–60 mg) are harmful. Nicotine induces both behavioral stimulation and anxiety in animals. Nicotine addiction involves drug-reinforced behavior, compulsive use, and relapse following abstinence. Nicotine withdrawal symptoms include depressed mood, stress, anxiety, irritability, difficulty concentrating, and sleep disturbances. Mild nicotine withdrawal symptoms are measurable in unrestricted smokers, who experience normal moods only as their blood nicotine levels peak, with each cigarette. The withdrawal symptoms worsen sharply and then gradually improve to a normal state. The evidence suggests that exposure to nicotine between the ages of 10 and 25 years causes lasting harm to the brain and cognitive ability (U.S. Department of Health and Human Services 2016). Nicotine use during pregnancy increases the child's risk of type 2 diabetes, obesity, hypertension, neurobehavioral defects, respiratory problems, and infertility (Schraufnagel et al. 2014).

All parts of the plant contain nicotine, which can be extracted and used as an insecticide. The dried leaves can also be used; they remain effective for 6 months after drying. The juice of the leaves can be rubbed on the body as an insect repellent. The leaves can be dried and chewed as an intoxicant. The dried leaves are also used as snuff or are smoked. This is the main species that is used to make cigarettes, cigars, and other products for smokers.

34.10 Amide Alkaloids

The chili pepper, also called chile, chile pepper, chilli pepper, or chilli, is the fruit of plants from the genus *Capsicum*, which are members of the nightshade family, Solanaceae. Chili peppers are widely used in many cuisines as a spice to add heat to dishes, usually in the form of spices such as chili powder and paprika. In high amount, chili pepper will cause a burning effect on other sensitive areas, such as the skin or eyes. Because people enjoy the heat, there has long been a demand for chili pepper products like curry, chili con carne, and hot sauces such as Tabasco sauce and salsa. Chili peppers originated in Mexico. After the Columbian exchange, many cultivars of chili pepper spread across the world, used for both food and traditional medicine. *Capsicum* cultivars belong to the five major species of cultivated peppers: *Capsicum annuum*, *Capsicum chinense*, *Capsicum baccatum*, *Capsicum frutescens*, and *Capsicum pubescens*. Cultivars grown in North America and Europe are believed to all derive from *Capsicum annuum* and have white, yellow, red, or purple to black fruits. In 2016, world production of raw green chili peppers was 34.5 million tons, with China producing half of the world's total production.

Capsaicinoids are the active components of chili peppers. The most commonly occurring capsaicinoids are capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%) (Fig. 10). Capsaicinoids are irritants for mammals, including humans, and produce a sensation of burning in any tissue with which they come into contact. Capsaicinoids function as deterrents against certain mammals and fungi. Pure capsaicin is a hydrophobic, colorless, highly pungent, and crystalline to waxy solid compound. Capsaicin and dihydrocapsaicin are the most pungent capsaicinoids; nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin are about half as hot.

Capsaicin is used as an analgesic in topical ointments and dermal patches to relieve pain, typically in concentrations between 0.025% and 0.1%. It may be applied in cream form for the temporary relief of minor aches and pains of

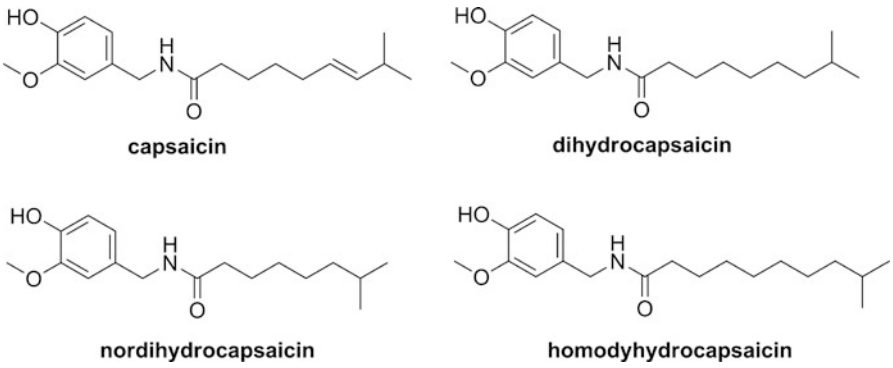


Fig. 10 Structure of representative capsaicinoids

muscles and joints associated with arthritis, backache, strains, and sprains, often in compounds with other rubefacients.

Capsaicin is also used to reduce the symptoms of peripheral neuropathy, such as postherpetic neuralgia caused by shingles. Capsaicin transdermal patch (Qutenza) for the management of this particular therapeutic indication (pain due to postherpetic neuralgia) was approved as a therapeutic by the US Food and Drug Administration, but a subsequent application for Qutenza to be used as an analgesic in HIV neuralgia was refused. It is common for people to experience pleasurable and even euphoric effects from ingesting capsaicin. Folklore among self-described "chiliheads" attributes this to pain-stimulated release of endorphins, a different mechanism from the local receptor overload that makes capsaicin effective as a topical analgesic.

The burning and painful sensations associated with capsaicin result from its chemical interaction with sensory neurons. Capsaicin binds to a receptor called the vanilloid receptor subtype 1 (TRPV1). By binding to the TRPV1 receptor, the capsaicin molecule produces similar sensations to those of excessive heat or abrasive damage, explaining why the spiciness of capsaicin is described as a burning sensation.

Capsaicin is a strong irritant requiring proper protective goggles, respirators, and proper hazardous material handling procedures. Capsaicin takes effect upon skin contact (irritant, sensitizer), eye contact (irritant), ingestion, and inhalation (lung irritant, lung sensitizer). LD_{50} in mice is 47.2 mg/kg. Painful exposures to capsaicin-containing peppers are among the most common plant-related exposures presented to poison centers. They cause burning or stinging pain to the skin and, if ingested in large amounts by adults or small amounts by children, can produce nausea, vomiting, abdominal pain, and burning diarrhea. Eye exposure produces intense tearing, pain, conjunctivitis, and blepharospasm. The primary treatment is removal from exposure. For external exposure, bathing the mucous membrane surfaces that have contacted capsaicin with oily compounds such as vegetable oil, paraffin oil, petroleum jelly (Vaseline), creams, or polyethylene glycol is the most effective way to attenuate the associated discomfort. Capsaicin can also be washed off the skin using soap, shampoo, or other detergents. When capsaicin is ingested, cold milk is an effective way to relieve the burning sensation; and room-temperature sugar solution (10%) at 20 °C (68 °F) is almost as effective. The burning sensation will slowly fade away over several hours if no actions are taken. Capsaicin-induced asthma might be treated with oral antihistamines or corticosteroids.

34.11 Conclusions

Many alkaloid-containing foods have certain physiological activities, such as the anti-pressure effect of lotus root, the anticancer effect of solanine, and the antibacterial effect of broccoli. Further chemical and pharmacological studies are definitely needed to elucidate the chemical principles of these foods and underlying mechanisms. Moreover, investigations to determine alkaloid amount in foods are of importance of controlling food safety, and quality has a decisive influence. For

example, morphine in poppy shells, nicotine in tobacco, total alkaloids in lotus leaves, and solanine in potatoes are all safe as food. The alkaloids in food have great potential for development as food supplements or drugs. For example, the alkaloids in tomatoes can be developed as natural food preservatives, and the caffeine in tea can replace some additives and drugs. A small amount of edible alkaloids is good for human health, and excessive amounts are harmful. A closer understanding of the alkaloids in food can lead to a healthier diet and better benefit for human beings.

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Dietary Coumarins

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Abstract

Dietary phytochemicals are bioactive components of plant-based foods (phyto-foods), e.g., fruits, vegetables, grains, and tea. Their regular and adequate intake may offer protection from major chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative diseases. Phyto-foods like apples, blueberries, broccoli, cherries, soybeans, and walnuts contain bioactive phytochemicals of various chemical classes, in addition to usual micronutrients and fibers. One of such major groups of phytochemicals found in plant-based food items are naturally occurring coumarins, which belong to the chemical class of 1-benzopyran derivatives. Plants from the families Apiaceae, Asteraceae, and Rutaceae are three major sources of naturally occurring coumarins. Some commonly consumed coumarin-containing phyto-foods include carrots, celery, parsnip, strawberries, citrus fruits, apricots, and cherries, as well as spices like cinnamon and fennels. This chapter presents a coverage primarily on most common coumarins that occur in food plants and their contributions to human health and wellbeing.

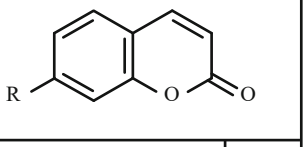
Keywords

Coumarins · Phenolics · Apiaceae · Asteraceae · Rutaceae · Bioactivity · Bioavailability · Food source · Plant · Marketed products · Patents

35.1 Introduction

Dietary phytochemicals are usually biologically active components of plant-based foods (sometimes referred to as "phyto-food"), e.g., fruits, vegetables, grains, and tea. Their regular and adequate intake often offers protection from major chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative diseases (Krzyzanowska et al. 2010; Probst et al. 2017; Bansal et al. 2018). Phyto-foods like apples, blueberries, broccoli, cherries, soybeans, and walnuts contain bioactive phytochemicals of various chemical classes, in addition to usual micronutrients and fibers (Dillard and German 2000). One of such major groups of phytochemicals found in plant-based food items is naturally occurring coumarins.

Coumarins are the largest class of 1-benzopyran derivatives ubiquitously distributed in the plant kingdom (Sarker and Nahar 2017). There are about 4000 naturally occurring coumarins identified to date. Coumarin (**1**) (2*H*-1-benzopyran-2-one) (Fig. 1), a fragrant colorless compound isolated from the tonka bean (*Dipteryx odorata*, family Fabaceae) in 1820, is the first member of the coumarin class. The



Coumarins	R
Coumarin (**1**)	H
Umbelliferone (**2**)	OH

Fig. 1 Structures of coumarin (**1**) and 7-hydroxycoumarin (umbelliferone, **2**)

name coumarin comes from a French term for the tonka bean, *coumarou*. The families Apiaceae, Asteraceae, and Rutaceae are three major sources of naturally occurring coumarins (Sarker and Nahar 2007). Some commonly consumed coumarin-containing phyto-foods include apricots (*Prunus armeniaca*), carrots (*Daucus carota*), celery (*Apium graveolens*), cherries (*Prunus avium*), citrus fruits (*Citrus* spp.), parsnip (*Pastinaca sativa*), and strawberries *(Fragaria* × *ananassa)*, as well as spices like aniseed (*Pimpinella anisum*), caraway (*Carum carvi*), cinnamon (*Cassia cinnamon*), coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), and fennel (*Foeniculum vulgare*) (Table 1).

Most natural coumarins possess oxygenation at C-7. Umbelliferone (7-hydroxycoumarin, **2**), first isolated from the family Umbelliferae (*alt.* Apiaceae), is the first member of the 7-oxygenated coumarins. It is the parent compound for the biosynthesis of all other highly oxygenated, prenylated, geranylated, farnesylated, and more complex forms of natural coumarin derivatives (Sarker and Nahar 2017).

The biosynthesis of natural coumarins often begins from *para*-coumaric acid (**3**, also known as *trans*-4-hydroxycinnamic acid) following the phenylpropanoid pathway (Fig. 2), and proceeds through an enzyme-mediated oxidation leading to the formation of 2-hydroxy-*para*-coumaric acid (**4**), which is then glycosylated to form its 2-glucoside (**5**) (Sarker and Nahar 2007, 2017). Intermediate **5** undergoes isomerization to form its *cis*-isomer (**7**), which finally produces umbelliferone (**2**) through ring closure.

Usually, the classification of natural coumarins is based on structural diversities that exist in this group of compounds and can be classified into simple, simple prenylated, simple geranylated, furano, pyrano, sesquiterpenyl, and oligomeric coumarins (Table 2) (Sarker and Nahar 2007).

In plants, coumarins contribute to the defense against phytopathogens, response to abiotic stresses, and regulation of oxidative stress and act as signaling molecules; they also possess considerable medicinal values, and many of these bioactive coumarins are found in dietary plants and plant components (Sarker and Nahar 2007). The major therapeutic applications of coumarins stem from their antimicrobial, antiviral, anti-inflammatory, antidiabetic, antioxidant, and various enzyme inhibitory activities. In addition to their therapeutic properties, the aromatic properties of coumarins, especially in spices, are exploited extensively in culinary, cosmetic, and tobacco industries. This chapter will restrict its coverage primarily to the

Table 1 Major food plants that contain coumarins

Plant families	Common names	Botanical names
Apiaceae (*alt.* Umbelliferae)	Anise	*Pimpinella anisum* L.
	Caraway	*Carum carvi* L.
	Carrot	*Daucus carota* L.
	Celery	*Apium graveolens* L.
	Coriander	*Coriandrum sativum* L.
	Dill	*Anethum graveolens* L.
	Parsley	*Petroselinum crispum* (Miller) Nyman ex A. W. Hill
	Parsnip	*Pastinaca sativa* L.
Moraceae	Fig	*Ficus carica* L.
Rutaceae	Grapefruit	*Citrus x paradisi* Macfad.
	Lemon	*Citrus limon* (L.) Burn. f.
	Lime	*Citrus aurantifolia* (Christm.) Swingle
	Orange	*Citrus sinensis* (L.) Osbeck

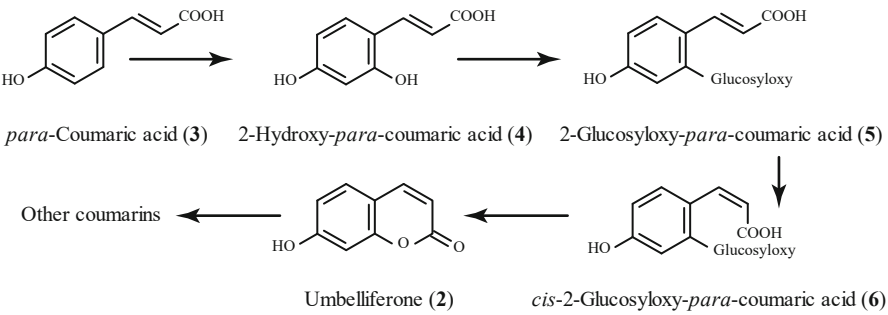


Fig. 2 Biosynthesis of natural coumarins

Table 2 Classification of coumarins

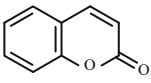
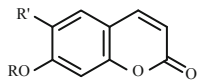
Coumarin classes	Core structures	Examples
Simple coumarins		 Chicoriin (**7**) R = glucosyl; R′ = OH; Esculetin (**8**) R = H; R′ = OH Scopoletin (**9**) R = H; R′ = OMe

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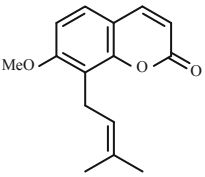
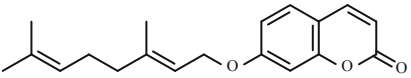
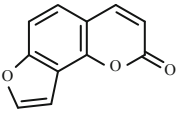
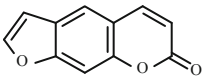
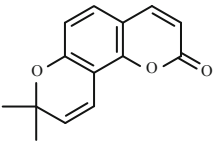
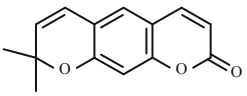
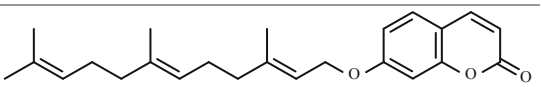
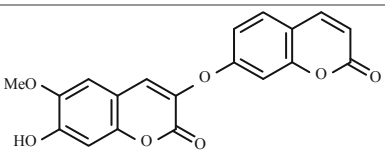
Table 2 (continued)

Coumarin classes	Core structures	Examples
Simple prenylated coumarins	One or more prenyl (C_5) or modified prenyl group on the core simple coumarin structure	 Osthol (**10**)
Simple geranylated coumarins	One or more geranyl (C_{10}) or modified geranyl group on the core simple coumarin structure	 Auraptene (**11**)
Furanocoumarins	One or more furan or dihydrofuran ring fused with the simple coumarin skeleton (angular or linear)	 Angelicin (**12**);  psoralen (**13**)
Pyranocoumarins	One or more pyran or dihydropyran ring fused with the simple coumarin skeleton (angular or linear)	 Seselin (**14**);  xanthyletin (**15**)
Sesquiterpenyl coumarins	One or more farnesyl (C_{15}), modified farnesyl, or a sesquiterpenyl group linked to the core simple coumarin structure	 Umbelliprenin (**16**)
Olegomeric coumarins	Two or more coumarin units linked or fused together	 Daphnoretin (**17**)

most common coumarins that occur in food plants and can be placed broadly under the category of dietary phytochemicals.

35.2 Bioactive Dietary Coumarins

Many coumarins found in food plants are bioactive, and they, being part of human diets, contribute to human health and well-being. Table 3 lists most commonly available dietary coumarins, their sources, and summarizes their reported bioactivities (mainly based on *in vitro* studies), and Figs. 3 and 4 show the structures of those coumarins.

Table 3 Most common dietary coumarins, their sources, and reported *in vitro* bioactivities

Dietary coumarins	Dietary sources	Bioactivities	References
Angelicin (**12**)	Parsnip	Anticancer, antioxidant, and antiviral	Wagstaff (1991), Cho et al. (2013), Wang et al. (2017a)
Auraptene (**11**)	Grapefruit	Acetylcholinesterase inhibitor, antimicrobial, and chemoprevention	Miyazawa et al. (2001)
Bergamottin (**29**)	Grapefruit, lemon, and lime	Anticancer, antioxidant, anti-inflammatory, and antitumor	Wagstaff (1991), Miyake et al. (1999), Dugrand et al. (2013), Hung et al. (2017)
Bergapten (**27**)	Aniseed, carrot, caraway, celery, coriander, dill, fennel, fig, grapefruit, lemon, parsley, and parsnip	Antimicrobial, inhibition of human cytochrome P450 1A2 (hCYP1A2) activity and hCYP1A2-mediated mutagenicity of aflatoxin B-1, and immunomodulation	Wagstaff (1991), Ozcelik et al. (2004), Peterson et al. (2006), Cherng et al. (2008), Rouaiguia-Bouakkaz et al. (2013)
Bergaptol (**28**)	Grapefruit and orange	Anti-inflammatory and antioxidant	Wagstaff (1991), Hung et al. (2017)
Byakangelicol (**37**)	Lemon	Anti-inflammatory and antioxidant	Dugrand et al. (2013)
Celerin (**24**)	Celery	Antimicrobial and antioxidant	Garg et al. (1980)
Coumarin (**1**)	*Cassia cinnamon* as a spice in food items and tonka bean (*Dipteryx odorata*) as food additives	Antitumor	Lacy and O'Kennedy (2004), Wang et al. (2013)
6′,7′-Dihydroxybergamottin (**36**)	Grapefruit	Anticancer, anti-inflammatory, and antioxidant	Hung et al. (2017)

(continued)

Table 3 (continued)

Dietary coumarins	Dietary sources	Bioactivities	References
Esculetin (**8**)	Coriander	Antiviral, inhibition of growth and cell cycle progression by inducing arrest of the G1 phase in HL-60 leukemia cells	Lacy and O'Kennedy (2004), Anesyan et al. (2007), Detsi et al. (2017)
8-Geranyloxypsoralen (**30**)	Lemon	Antitumor	Miyake et al. (1999), Dugrand et al. (2013)
5-Geranyloxy-7-methoxycoumarin (**26**)	Lemon and lime	Anticancer and antitumor	Miyake et al. (1999), Dugrand et al. (2013), Patil et al. (2013)
4-Hydroxycoumarin (**21**)	Coriander	Anti-inflammatory and antioxidant	Anesyan et al. (2007)
Imperatorin (**34**)	Lime and parsley	Anti-inflammatory and antioxidant	Wagstaff (1991)
Isofraxidin (**19**)	Celery	Anti-inflammatory, antilipidemic, and antioxidant	Li et al. (2017a)
Isoimperatorin (**38**)	Lime, lemon, and parsley	Anti-inflammatory and antioxidant	Wagstaff (1991), Dugrand et al. (2013)
Isopimpinellin (**31**)	Carrot, celery, fennel, grapefruit, lime, and parsnip	Antimycobacterial and immunomodulatory	Wagstaff (1991), Cherng et al. (2008), Hung et al. (2017)
Limettin (**25**)	Grapefruit, lemon, and lime	Antibacterial and anticancer	Dugrand et al. (2013), Patil et al. (2013)
Marmesin (**41**)	Lime	Anti-inflammatory, antimicrobial, and antioxidant	Shalaby et al. (2011)
Osthol (**10**)	Grapefruit and lemon	Anticancer, anti-inflammatory, antimicrobial, and antioxidant	Dugrand et al. (2013), Zhu et al. (2018b)
Oxypeucedanin (**39**)	Dill, lemon, and parsley	Antimycobacterial	Wagstaff (1991), Stavri and Gibbons (2005), Chavez et al. (2011), Dugrand et al. (2013)
Oxypeucedanin hydrate (**40**)	Dill, lemon, lime, parsley, and parsnip	Antimycobacterial	Wagstaff (1991), Stavri and Gibbons (2005), Chavez et al. (2011), Dugrand et al. (2013)
Phellopterin (**35**)	Lime	Antibacterial	Wagstaff (1991)

(continued)

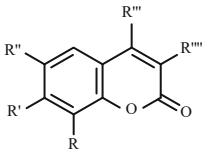
Table 3 (continued)

Dietary coumarins	Dietary sources	Bioactivities	References
Psoralen (**13**)	Aniseed, carrots, celery, fig, parsley, and parsnips	Inhibition of human cytochrome P450 1A2 (hCYP1A2) activity and hCYP1A2-mediated mutagenicity of aflatoxin B-1	Wagstaff (1991), Peterson et al. (2006), Rouaiguia-Bouakkaz et al. (2013)
Scoparone (**22**)	Lime, lemon, and orange	Anti-inflammatory, antimicrobial, antioxidant, and antiviral	Tatum and Berry (1977), Detsi et al. (2017)
Scopoletin (**9**)	Gari, a cassava food consumed in Nigeria, and traditional herbal infusion of the fruits of *Tetrapleura tetraptera*	Antimicrobial, antioxidant, antiviral, hypotensive, and non-specific spasmolytic agent	Obidoa and Obasi (1991), Detsi et al. (2017)
Scopolin (**23**)	Grapefruit	Anti-inflammatory, antioxidant, and antiviral	Runkel et al. (1997), Detsi et al. (2017)
Seselin (**14**)	Celery	Antihyperlipidemic and antitumor	Iyer and Patil (2018)
Umbelliferone (**2**)	Carrot, caraway, celery, dill, grapefruit, and lemon	Antimicrobial, antioxidant, antitumor antiviral, chemoprevention, and inhibition of release of cyclin D1	Lacy and O'Kennedy (2004), Ozcelik et al. (2004), Dhalwal et al. (2008), Dugrand et al. (2013), Najda et al. (2015), Detsi et al. (2017)
Umbelliprenin (**16**)	Celery, coriander, and lemon	Anticancer, anti-inflammatory, antileishmanial, antioxidant, and antitumor	Wang et al. (2019a)
Xanthotoxin (**32**)	Aniseed, carrot, caraway, celery, coriander, dill, fennel, fig, parsley, and parsnip	Antimicrobial, inhibition of human cytochrome P450 1A2 (hCYP1A2) activity and hCYP1A2-mediated mutagenicity of aflatoxin B-1, and immunomodulatory	Wagstaff (1991), Ozcelik et al. (2004), Peterson et al. (2006), Cherng et al. (2008), Rouaiguia-Bouakkaz et al. (2013)

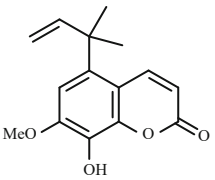
(continued)

Table 3 (continued)

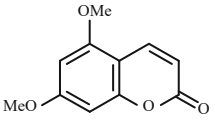
Dietary coumarins	Dietary sources	Bioactivities	References
Xanthotoxol (**33**)	Parsnip	Antimicrobial and antioxidant	Yu et al. (2017)
Xanthyletin (**15**)	Orange	Anti-inflammatory	Tatum and Berry (1977), Chan et al. (2010)



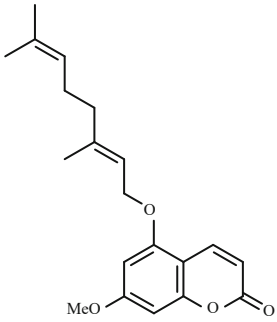
	R	R’	R’’	R’’’	R’’’’
Esculin (**18**)	H	OH	Glucosyloxy	H	H
Isofraxidin (**19**)	OMe	OH	OMe	H	H
3-Hydroxycoumarin (**20**)	H	H	H	H	OH
4-Hydroxycoumarin (**21**)	H	H	H	OH	H
Scoparone (**22**)	H	OMe	OMe	H	H
Scopolin (**23**)	H	Glucosyloxy	OMe	H	H



Celerin (**24**)



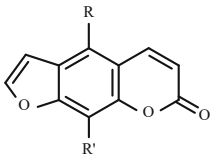
Limettin (**25**)



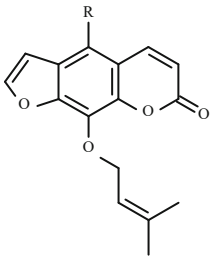
5-Geranyloxy-7-methoxycoumarin (**26**)

Fig. 3 Structures of simple, prenylated, and geranylated coumarins present in food plants

Most of these dietary coumarins are furanocoumarins, and mainly from the food plants of the family Apiaceae. For example, grapefruit furanocoumarins, e.g., 6′,7′-dihydroxybergamottin (**36**), bergamottin, (**29**) and bergapten (**27**), possess antioxidant, anti-inflammatory, anticancer, and bone health-promoting properties. They also exert antiproliferative activities against cancer cell growth through modulation of several molecular pathways, such as regulation of the



	R	R’
Bergapten (**27**)	OMe	H
Bergaptol (**28**)	OH	H
Bergamottin (**29**)	Geranyloxy	H
8-Geranyloxypsoralen (**30**)	H	Geranyloxy
Isopimpinellin (**31**)	OMe	OMe
Psoralen (**13**)	H	H
Xanthotoxin (**32**)	H	OMe
Xanthotoxol (**33**)	H	OH



	R
Imperatorin (**34**)	H
Phellopterin (**35**)	OMe

Fig. 4 (continued)

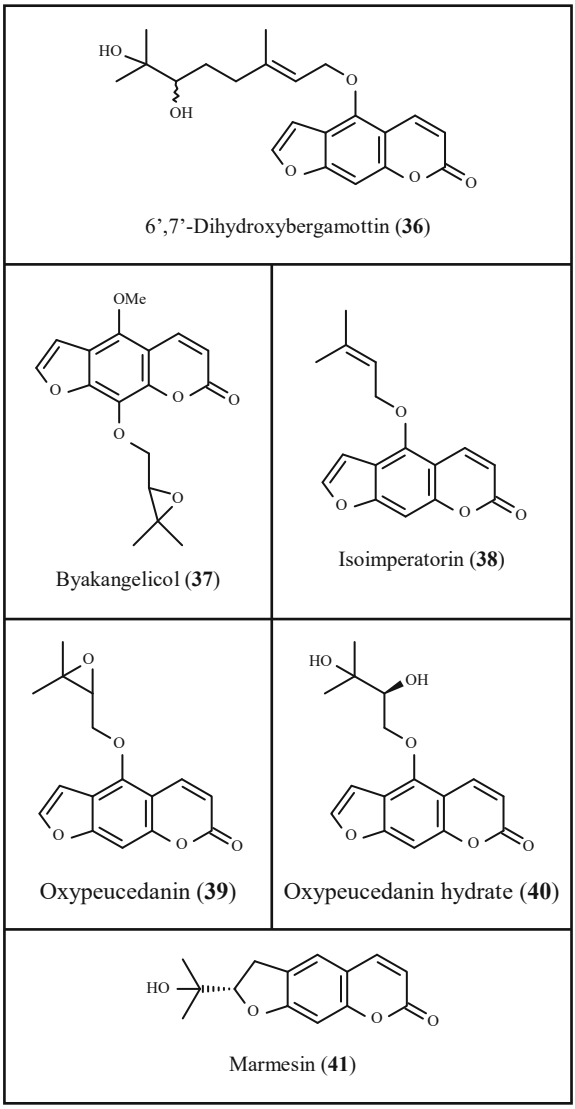


Fig. 4 Structures of furano and dihydrofurano coumarins present in food plants

signal transducer and activator of transcription 3, nuclear factor-κB (NF-κB), phosphatidylinositol-3-kinase/protein kinase B (AKt), and mitogen-activated protein kinase expression (Hung et al. 2017). However, several reports on *in vitro* bioactivities of various *Citrus* coumarins (family: Rutaceae) are also available. For example, bergamottin (**29**), and 5-geranyloxy-7-methoxycoumarin (**26**) and 8-geranyloxypsolaren (**26**) from *Citrus limon* were reported to possess

antitumor activity (Miyake et al. 1999). Similarly, 5-geranyloxy-7-methoxycoumarin (**26**), limettin (**25**), and isopimpinellin (**31**), from *Citrus aurantifolia* (lime), were shown to inhibit human colon cancer (SW-480) cell proliferation, with 5-geranyloxy-7-methoxycoumarin (**26**) being the most active (Patil et al. 2013).

As the discussion on *in vitro* bioactivity of dietary coumarins is not really within the remit of this chapter, the focus will be on the *in vivo* studies involving either animal models or human trials.

35.3 Bioavailability and Metabolism

Bioavailability refers to the degree and rate at which an administered drug is absorbed by the body's circulatory system, the systemic circulation. Thus, the effectiveness of any medication administered through the oral route depends on how quickly and how much of it is available in the blood circulation and whether the amount is at the therapeutic concentration. Bioavailability is an essential measurement tool, as it determines the correct dosage for non-intravenous administration of a drug. In clinical trials, the bioavailability of a drug is a key factor to be measured in Phase 1 and Phase 2 trials. Solubility and effective absorption are two major factors that control bioavailability of any oral dosage form. There are several studies on bioavailability and metabolism of some of the dietary coumarins, mainly in animal models, available to date.

The absorption and distribution of coumarin (**1**) is rather rapid through the gastrointestinal (GI) tract, despite poor water solubility of both coumarin (**1**) and umbelliferone (**2**) (Lacy and O'Kennedy 2004). However, because of high partition coefficients of these compounds, 21.5% for coumarin (**1**) and 10.4% for umbelliferone (**2**), the compounds are rapidly and almost completely absorbed once they are in aqueous solution, and owing to their non-polarity, they cross lipid bilayers easily by passive diffusion.

Pharmacokinetic studies involving humans confirmed that coumarin (**1**) is fully absorbed from the GI tract after oral administration and metabolized by the liver in the first pass with only 2–6% reaching the systemic circulation intact. The low bioavailability of coumarin (**1**), in addition to its short half-life (1.02 h per oral vs. 0.8 h intravenous), indicated that it is actually a prodrug, with its metabolite umbelliferone (**2**) being the compound of main pharmacological relevance. Note that a prodrug is a biologically inactive compound that can be metabolized in the body to produce a drug.

The metabolism of coumarin (**1**) itself has been studied quite extensively in various *in vitro* and *in vivo* models. Coumarin (**1**) is metabolized through hydroxylation primarily by specific cytochrome P450-linked monooxygenase enzyme (CYP2A6) system in the liver microsomes leading to the formation of **2**, which then undergoes Phase 2 conjugation reaction that produces glucuronide conjugate of **2**. Although hydroxylation is possible at all six positions (carbon atoms 3, 4, 5, 6, 7, and 8) in coumarin (**1**), the most common routes of hydroxylation are at C-7 and C-3,

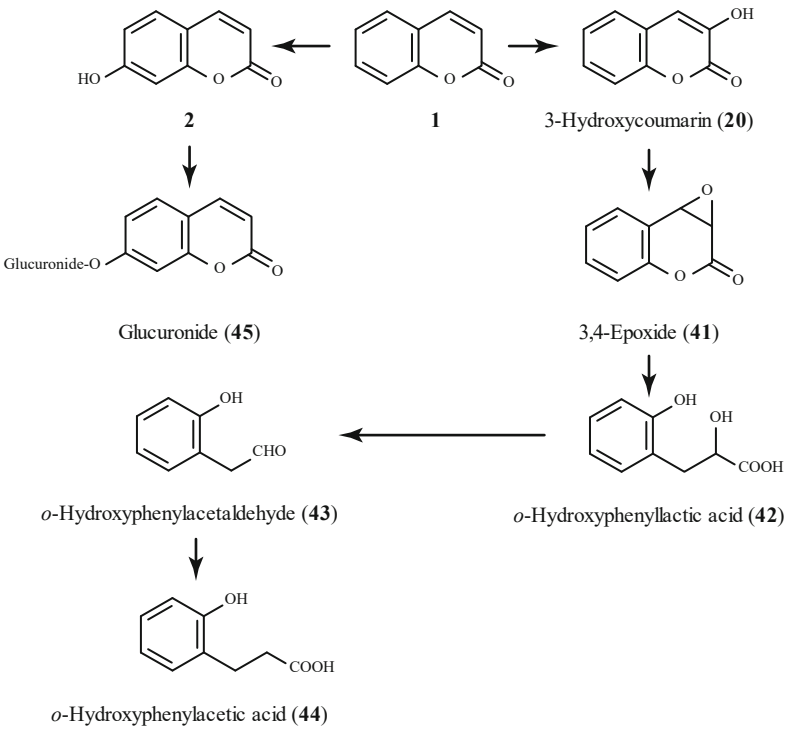


Fig. 5 Common routes of metabolism of coumarin (**1**). (Adapted from Lacy and O'Kennedy 2004)

yielding umbelliferone (**2**) and 3-hydroxycoumarin (**20**), respectively (Lacy and O'Kennedy 2004) (Fig. 5). 3-Hydroxylation leads to further metabolism *via* ring opening, producing two further products, *o*-hydroxyphenyllactic acid (*o*-HPLA, **42**) and *o*-hydroxyphenylacetic acid (*o*-HPAA, **44**). In humans, coumarin (**1**) is extensively and almost exclusively metabolized to umbelliferone (**2**). However, some individuals can metabolize a significant amount of coumarin (**1**) utilizing pathways other than 7-hydroxylation, e.g., the 3,4-epoxidation pathway to *o*-HPAA.

The absorption and metabolism of dietary furanocoumarins are well studied. Ingestion of, or dermal contact with, furanocoumarin-containing plants enable their absorption into the bloodstream (Melough et al. 2018). However, the kinetics and bioavailability of furanocoumarins can be quite variable depending on the dietary sources, as the matrix dictates dissolution, stability, gastric emptying, and intestinal absorption. In both humans and animals, ingested dietary furanocoumarins are rapidly absorbed in the GI tract; they go to systemic circulation and are taken up in the tissues in addition to the brain, the spleen, adipose, the kidneys, and the testis following an oral administration. Furanocoumarins could undergo extensive metabolism in the body; it was shown that the metabolism of these compounds began in the GI tract through the activity of intestinal bacteria or in the liver by cytochrome P450-dependent monooxygenases. It was suggested that 6′,7′-dihydroxybergamottin

(**36**) might be partially metabolized to bergaptol (**28**) before excretion in the urine (Melough et al. 2018). Although the exact pathways of furanocoumarin metabolism in humans have not yet been established conclusively, a few studies demonstrated that furanocoumarin metabolites were excreted in urine as hydroxylated products or in the form of glucuronides.

Bioavailability and metabolism of scopoletin (**9**) was studied in a dog model after intravenous (1 mg/kg) and oral (10, 25 and 50 mg/kg) administrations (Zhao et al. 2018a); it had short elimination half-life, its oral bioavailability was low (~6–7%), and glucuronidation was the predominant metabolic pathway. A similar study was performed with three simple coumarins, scopolin (**23**), scopoletin (**9**), and isofraxidin (**19**), in a mice model (Zhang et al. 2018a), where it was observed that the metabolism of these coumarins involved multiple *in vivo* metabolic reactions including glucuronidation, sulfonation, isomerism, and reduction. Glucuronidation was found to be the major route for xanthotoxol (**33**) metabolism in human (He et al. 2018).

The oral bioavailability of auraptene (**11**) was observed to be 8.5% after oral administration in rats, as measured by an LC-MS/MS method (Ye et al. 2016). After auraptene (100 mg/kg, p.o.) administration, the maximum plasma concentration and the time taken to reach maximum concentration were 1719.5 ± 384.3 g/mL and 108.0 ± 25.3 min, respectively, and the elimination half-life was 108.0 ± 25.3 for the dose 100 mg/kg, p.o., and 3.0 ± 0 min for 2 mg/kg, i.v.

The bioavailability and metabolism of bergapten (**27**), a well-known anti-inflammatory and antitumour natural furanocoumarin, was studied after oral administration (15 mg/kg) in a rat model (Yu et al. 2016). The absolute oral bioavailability (F) were 80.1 ± 29.6%, 94.0 ± 40.3%, and 69.5 ± 44.2% for low, medium, and high concentrations using the formula F = (AUC oral/AUC intravenous) × 100%, based on the AUC(0–∞) obtained after i.v. and oral administrations. The AUC of the medium and high concentration were similar, which meant that the absorption of **27** reached its peak within the range of 10–15 mg/kg. Bergapten (**27**) might be well absorbed from the GI tract in rat, and an oral administration of this coumarin might be the better route for clinical use.

35.4 Bioactivities (Animal Studies)

Although various chemical, cell line, and enzymatic *in vitro* assays dominate the bioactivity assessment arena, there are significant amounts of bioactivity data from various *in vivo* studies involving several animal models available on dietary coumarins. Some major bioactivities of dietary coumarins as evident from *in vivo* studies include analgesic, anti-allergy, anti-asthma, anticancer, anticolitis, antidepressant, antidiabetic, antidermatitis, antihypertensive, anti-inflammatory, anti-obesity, anti-osteoporotic, anti-pruritic, antipsoriatic, antipsychotic, antitumor, cardioprotective, gastroprotective, hepatoprotective, nephroprotective, and neuroprotective activities, and they are discussed below.

35.4.1 Analgesic

An analgesic or painkiller is a substance that produces analgesia (relief from pain). Analgesic agents act in several ways on the peripheral and central nervous systems (CNS). Among the most common dietary coumarins, imperatorin (**34**), osthol (**10**), seselin (**14**), and xanthotoxol (**33**) possess considerable analgesic property. The analgesic potential of **10** was shown in acetic acid and formalin-induced hyperalgesia in mice (Singh et al. 2018). Osthol (**10**), administered epidurally in the early stage of pain in rats, could alleviate the pain for a long time, which might be attributed by the inhibition of p38 mitogen-activated protein kinase signaling-related pathways (Zhang et al. 2018c). Analgesic activity of **14** was studied in a mice model (Lima et al. 2006). This coumarin could inhibit inflammatory hyperalgesia, suggesting that seselin (**14**) might possesses both important peripheral anti-inflammatory and antinociceptive properties. Imperatorin (**34**) and xanthotoxol (**33**) displayed analgesic property, mediated through the central opioidergic system, in an *in vivo* study involving rats (Kumatia et al. 2017).

35.4.2 Anticolitis

Colitis is the inflammation of the inner lining of the colon, often caused by infection, inflammatory bowel disease (Crohn's disease, ulcerative colitis), and allergic reactions. Coumarins **10** and **34** possess anticolitis property. The therapeutic potential of **10** in the treatment of colitis was reported in dinitrobenzene sulfonic acid-induced colitis in rats through its anti-inflammatory and antioxidant properties (Khairy et al. 2018). This coumarin improved the loss in body weight, accompanied with betterment of the disruption of the colonic architecture as well as improvement in the antioxidant defense system. Treatment with osthol (**10**) could suppress activities on pro-inflammatory Th2-related cytokines and upregulation of anti-inflammatory Th2-related cytokines. Previously, Sun et al. (2017) demonstrated the anticolitis potential of **10**. Pretreatment with this compound could alleviate trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice by reducing the expression of inflammatory mediators and attenuating p38 phosphorylation *via* both cAMP/PKA (cyclic AMP/protein kinase A)-dependent and independent pathways, among which the cAMP/PKA-independent pathway played a major role. Pretreatment with **10** also ameliorated the clinical scores, colon length shortening, colonic histopathological changes, the expression of inflammatory mediators in TNBS-induced colitis, and increased serum cAMP levels. The anticolitis property of imperatorin (**34**) was shown in a study in a dextran sulphate sodium-induced colitis mouse model Liu et al. (2018d); coumarin **34** could activate CYP3A4 promoter activity, recruit steroid receptor ac-activator 1 to the ligand binding domain of pregnane X receptor, and increase the expression and activity of CYP3A4.

35.4.3 Antidermatitis, Antipruritic, and Antipsoriatic

Psoriasis is a skin condition that causes red, flaky, crusty patches of skin covered with silvery scales. These patches normally appear on elbows, knees, scalp, and lower back but can appear anywhere on the body. Most people are only affected with small patches, and in some cases, the patches can be itchy or sore. Chen et al. (2018a) investigated the effects of esculetin (**8**) on psoriatic skin inflammation in a mice model and studied its plausible molecular mechanisms of action to conclude that coumarin **8** could attenuate psoriasis-like skin lesion in mice and might possess clinical significance. Jeong et al. (2018) reported a similar observation, where compound **8** attenuated atopic skin inflammation by inhibiting the expression of inflammatory cytokines, suggesting its potential therapeutic applications in the treatment of atopic dermatitis.

The antidermatitis and antipruritic potential of osthol (**10**) is well documented. Sun et al. (2018) established that the antipruritic effect of **10** could be mediated by selective inhibition of thermosensitive transient receptor potential vanilloid 3 channel in the skin in mice model.

Psoralen (**13**) and its derivatives together with ultraviolet (UV) lights could be useful in reducing psoriatic response, both in murine and human models. The effect of bergapten (**27**), which is actually 8-methoxypsoralen, plus UV A or UV B alone on imiquimod-induced psoriasis was examined in a mice model (Shirsath et al. 2018). Mouse skin was treated with repetitive sub-phototoxic doses of **27** plus UV A or UV B alone before or during the induction of toll-like receptor 7/8 activation and psoriasis through the application of imiquimod. Bergapten (**27**) plus ultraviolet A suppressed the established imiquimod-induced psoriatic phenotype to a greater degree than UV B alone, but pretreatment with the former, prior to administration of imiquimod, also reduced the susceptibility of murine skin to respond to imiquimod to a greater degree than did pretreatment with UV B alone.

35.4.4 Antidiabetic

Diabetes is a lifelong condition that causes an extremely high blood sugar level. There are two main types of diabetes: type 1 diabetes, where the body's immune system attacks and destroys the cells that produce insulin, whereas in type 2 diabetes, the body does not produce enough insulin or the body's cells do not react to insulin. However, between these two types, type 2 diabetes is far more common in the human populations. Dietary coumarins auraptene (**11**), esculetin (**8**), esculin (**18**), osthol (**10**), scopoletin (**9**), phellopterin (**35**), and umbelliferone (**2**) possess antidiabetic potential, mainly because of their antioxidative property. Coumarins **10** and **18** could improve type 2 diabetes in high-fat-diet- and streptozotocin-induced diabetic mice through antioxidative mechanisms (Yao et al. 2018a). Both coumarins lowered fasting blood glucose and blood lipid levels while increasing insulin levels. They exhibited antioxidant effects in the pancreas through elevating the activities of the enzymes glutathione peroxidase, catalase, and superoxide dismutase. Osthol (**10**) attenuated cellular imbalance, blurry fringes of hepatic sinusoid and extensive

vacuolization due to hepatocellular lipid accumulation, and reduced inflammatory infiltration in the pancreas.

Esculin (**18**) ameliorated cognitive improvement in experimental diabetic nephropathy, mediated through its anti-inflammatory and antioxidant actions (Song et al. 2018). Earlier, Kang et al. (2014) reported the protective effect of this compound on streptozotocin-induced renal damage in diabetic mice. Similarly, umbelliferone (**2**) was shown to protect liver injury in diabetic mice (Yin et al. 2018) and renal injury in type 1 diabetic rats (Garud and Kulkarni 2017) and to attenuate unpredictable chronic mild stress induced-insulin resistance in rats (Su et al. 2016). Although antihyperglycemic property of **2** is well known, Naowaboot et al. (2015) demonstrated that **2** could also improve impaired metabolism of glucose and lipid in high-fat-diet-/streptozotocin-induced type 2 diabetic rats. This action is mediated through inducing expression of adiponectin, which is a protein hormone involved in regulating glucose levels as well as fatty acid breakdown, glucose transporter type 4 (GLUT4, the insulin-regulated glucose transporter found primarily in adipose tissues and striated muscle), peroxisome proliferator-activated receptors (PPARs), and PPAR-protein expressions.

Esculetin (**8**) is equally effective against type 2 diabetes. Kadakol et al. (2017b) showed that **8** could ameliorate insulin resistance and type 2 diabetic nephropathy through reversal of histone H3 acetylation and H2A lysine 119 monoubiquitination. Choi et al. (2016) reported the effects and mechanism of **8** on nonalcoholic fatty liver in diabetic mice fed with high-fat diet and suggested that compound **8** could protect against development of nonalcoholic fatty liver in diabetes via regulation of lipids, glucose, and inflammation. The antidiabetic effect of scopoletin (**9**), which regulates glucose metabolism, was confirmed in a streptozotocin-induced mice model, particularly focusing on its effect on carbohydrate metabolizing enzymes, and postprandial hyperglycemia. This coumarin lowered postprandial hyperglycemia through inhibition of carbohydrate digestive enzymes, e.g., α-glucosidase and α-amylase (Jang et al. 2018). Kalaivanan et al. (2018a) exhibited that **9** intervention could suppress pancreatic endoplasmic reticulum stress (normally observed in type 2 diabetes sufferers) induced by lipotoxicity and revealed its potential in delaying the progression of insulin resistance. The same group also showed that supplementation of **9** could improve insulin sensitivity in rats by attenuating the imbalance of insulin signaling through 5′-adenosine-monophosphate-activated protein kinase (Kalaivanan et al. 2018b). Choi et al. (2017) investigated the effects of **9** on hepatic steatosis and inflammation in a high-fat-diet-fed type 1 diabetic mice in comparison with antidiabetic drug metformin. Both scopoletin (**9**) and metformin lowered blood glucose and hemoglobin A1c (HbA1c), serum alanine aminotransferase (ALT), tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) levels, glucose intolerance, and hepatic lipid accumulation compared with the diabetic control group. Both compounds downregulated hepatic gene expression of triglyceride and cholesterol synthesis as well as inflammation and upregulated cholesterol 7α-monooxygenase (CYP7A1) gene. They downregulated hepatic PPAR and diacylglycerol-*O*-acyltransferase 2 (DGAT2) protein levels and inhibited hepatic fatty acid synthase and phosphatidate phosphohydrolase activities suggesting that **9** could protect against diabetes-induced steatosis and inflammation by inhibiting lipid biosynthesis

and myeloid differentiation primary response 88 (TLR4-MyD88) pathways. Coumarin **9** could also protect against methylglyoxal-induced hyperglycaemia and insulin resistance mediated by suppression of advanced glycation end product generation and antiglycation (Chang et al. 2015). It can be noted that glycation is a non-enzymatic condensation reaction between reducing sugars and amino groups of proteins that undergo rearrangements to stable ketoamines, leading to the formation of advanced glycation end products.

Auraptene (**11**) regulates gene expression involved in lipid metabolism through PPAR-α activation in diabetic obese mice (Takahashi et al. 2011). In high-fat-diet-fed diabetic obese mice, this compound suppressed hyperlipidemia and triglyceride accumulation in the liver and skeletal muscle and increased the mRNA expression levels of the PPAR-α target genes involved in fatty acid oxidation in the liver and skeletal muscle. Furthermore, in the auraptene-treated mice, the adipocyte size was considerably smaller than that in the control high-fat-diet-fed mice rendering the improvement of high-fat-diet-induced hyperglycaemia and abnormalities in glucose tolerance.

The antidiabetic potential of phellopterin (**35**) was demonstrated *in vivo* using a diabetic mouse model (Han et al. 2018), where diabetes was induced by high-fat diets and streptozotocin. Treatment with **35**, at the doses of 1 mg/kg and 2 mg/kg, reduced the levels of blood glucose, triglycerides, and total cholesterol. It also enhanced adipocyte differentiation in preadipocytes. The study concluded that this coumarin might be a valuable therapeutic alternative for enhancing insulin sensitivity through promotion of adipocyte differentiation and by increasing mRNA expression levels of PPAR-γ, which is a major mediator of insulin sensitivity.

35.4.5 Antifertility

Two furanocoumarins, bergaptol (**28**) and xanthotoxin (**32**), showed antifertility activity in rodents (Ulubelen et al. 1994). About 70% of the test animals developed cystic and atretic follicles in their ovaries, and glomerulocapsular adhesion and segmental fusion were noticed in the kidneys. However, no adverse effect was observed in the brain.

35.4.6 Antihypertensive

Hypertension or high blood pressure is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure typically does not cause symptoms, but it puts extra strain on blood vessels, the heart, and other organs, such as the brain, the kidneys, and the eyes. Persistent high blood pressure can increase the risk of various serious and potentially life-threatening conditions, e.g., heart disease. Auraptene (**11**), imperatorin (**34**), osthol (**10**), and scopoletin (**9**) have antihypertensive property. Scopoletin (**9**), which is 6-methyl ether of esculetin (**8**), showed antihypertensive effect in an acute hypertension

animal model (Lagunas-Herrera et al. 2019). Antihypertensive effect of osthol (**10**) was demonstrated against pulmonary arterial hypertension, which is a progressive cardiovascular disease with high mortality (Yao et al. 2018c). Auraptene (**11**) displayed antihypertensive effect in a hypertensive rat model (Razavi et al. 2015); this compound reduced mean systolic blood pressure in hypertensive rats, but not in normotensive rats. The antihypertensive potential of imperatorin (**34**) was demonstrated in rats (Cao et al. 2013), where the 2-kidney, 1-chip (2 K,1C) model of hypertension was utilized.

35.4.7 Anti-inflammatory

An inflammation is a localized physical condition or state where part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection. Generally, diets containing high levels of antioxidants can minimize inflammation and somewhat act as anti-inflammatory agents. Several dietary coumarins possess anti-inflammatory property, which has been shown in various animal studies. The *in vivo* anti-inflammatory activity of umbelliferone (**2**), the most common natural coumarin, has recently been reported by Wang et al. (2019b), showing the protective effect and underlying mechanism of action of **2** against lipopolysaccharide (LPS)-induced acute lung injury, following the similar mechanism as stated earlier. However, in fact, several researchers previously demonstrated the *in vivo* anti-inflammatory and antioxidant effects of **2**, and associated mechanisms of actions were also studied quite extensively in various animal models (Rauf et al. 2014; Anwar et al. 2015; Sim et al. 2015; Germoush et al. 2018).

The role of esculin (**18**) against macrophages and endotoxin shock induced by LPS in mice was analyzed by Li et al. (2016a), where they showed that this glycoside could suppress inflammatory reactions in macrophages and protected mice from LPS-induced endotoxin shock. Pretreatment with **18** improved the survival rate of mice. It inhibited LPS-induced increase of TNF-α and IL-6 in serum, the lungs, the liver, and the kidneys and upregulated IL-10, an anti-inflammatory cytokine. Esculin (**18**) attenuated the tissue injury of the lungs, the liver, and the kidneys in endotoxic mice. It diminished the protein expression of NF-κB p65 in the lungs, the liver, and the kidneys, resulting in lowering of levels of inflammatory mediators. Earlier, Zhang and Wang (2015) reported anti-inflammatory effects of this coumarin on LPS-induced acute lung injury in a rat model. The protective effect of **18**, mediated though anti-inflammatory and antioxidant mechanisms, on hyperoxia-induced lung injury model in newly born rats was established (Qi and Yu 2016). Niu et al. (2015a) reported the anti-inflammatory activity of **18** in xylene-induced mouse ear edema, carrageenan-induced rat paw edema, and carrageenan-induced mouse pleurisy models, while in the same year, Zheng et al. (2015) highlighted the anti-inflammatory effects of **18** on the adjuvant-induced arthritis in adult female Sprague Dawley rats. In the latter study, the treatment with **18** improved the body weight of rats accompanied with a reduction of paw volume in comparison to arthritic control.

Esculetin (**8**), the aglycone of the coumarin glycoside esculin (**18**), could attenuate LPS-induced neuro-inflammatory processes and depressive-like behaviour in mice (Zhu et al. 2016). It could reduce LPS-induced elevated levels of pro-inflammatory cytokines including IL-6, interleukin-1β (IL-1β), and TNF-α in serum and hippocampus. It also attenuated nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expressions by inhibiting NF-kappa B (NF-κB) pathway in hippocampus. Rzodkiewicz et al. (2016) showed that **8** could reduce leukotriene B4 level in plasma of rats with adjuvant-induced arthritis, indicating its potential as a drug candidate for the treatment of rheumatoid arthritis. In a rat experimental model of inflammatory bowel disease induced by trinitrobenzenesulfonic acid, coumarin **8** at the dose of 5 mg/Kg exhibited intestinal anti-inflammatory activity (Witaicenis et al. 2014).

Another simple coumarin, scopoletin (**9**), could offer protection against cerulein-induced acute pancreatitis and associated lung injury in mice; the effect was because of its anti-inflammatory effect mediated through downregulation of pre-protachykinin A expression (encodes neuropeptide substance P) in the pancreas and the lungs and hydrogen sulfide signaling *via* nuclear factor B pathway (Leema and Tamizhselvi 2018). The anti-inflammatory property of **9** was shown *in vivo* in various other animal studies (Choi et al. 2017; Osman et al. 2017). Almost in all those cases, the anti-inflammatory activity of **9** was mediated through decreasing the myeloperoxidase and adenosine-deaminase activities and nitric oxide, TNF-α, and IL-1β levels and could be attributed to its ability to inhibit the phosphorylation of NF-κB and p38 MAPK (mitogen-activated protein kinases). Earlier, a similar observation was made with isofraxidin (**19**) in a xylene-induced mouse ear edema model (Niu et al. 2012); this coumarin exhibited anti-inflammatory effects *in vivo* and inhibited TNF-α production in LPS-induced mouse peritoneal macrophages *in vitro via* the mitogen-activated protein kinases pathway. Scopolin (**23**), the glucoside of **9**, was shown to suppress adjuvant-induced rat arthritis by inhibiting inflammation and angiogenesis at a dose of 100 mg/kg (Pan et al. 2009).

The anti-inflammatory potential of osthol (**10**) was exhibited in carrageenan-induced paw edema in mice (Singh et al. 2018); involvement of nitric oxide (NO) and cyclooxygenase pathways was investigated, the expression of inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) was determined in spinal sections by immunohistochemical analysis, and LPS-challenge was used to assess *in vivo* effect on inflammatory cytokines. Osthol (**10**) could decrease carrageenan-induced paw edema, the levels of iNOS and COX-2 expression as well as TNF-α and IL-6. While the immunosuppressive effect of **10** is known, its potential as an anti-inflammatory agent was further established by Xu et al. (2018) in a collagen-induced arthritis rat model. This coumarin was effective on uric acid crystal-induced acute gouty arthritis through the inhibition of inflammasome, which is a multi-protein intracellular complex that detects pathogenic microorganisms and sterile stressors and that activates the highly pro-inflammatory cytokines IL-1β and IL-18. It can be mentioned that inflammasomes also induce a form of cell death termed pyroptosis (Yang et al. 2018a). The anti-inflammatory therapeutic potential of osthol (**10**) has been demonstrated in a number of other studies involving various *in vivo* animal models

(Bao et al. 2018; Jin et al. 2018; Khairy et al. 2018; Wu et al. 2018a; Zhang et al. 2018c; Zhao et al. 2018c).

Auraptene (**11**) is known to exert immunomodulatory effect and shows anti-inflammatory effects in peripheral tissues, and this was demonstrated in the brains of a LPS-injected systemic inflammation animal model and a brain ischemic mouse model (Okuyama et al. 2016, 2018). Seselin (**14**), an angular pyranocoumarin found in celery, has recently been shown to exert anti-inflammatory activity in two models of sepsis, cecal ligation and puncture or injection of LPS, in C57BL/6 mice, which is a common inbred strain of laboratory mice (Feng et al. 2019). It can also be noted that the C57BL/6 mouse was the second-ever mammalian species to have its entire genome published. This coumarin ameliorated sepsis induced by cecal ligation and puncture and produced anti-inflammatory activity through its action on Janus kinase 2 (JAK2), which is a kinase that is mutated in a number of myeloproliferative diseases and cancers. Earlier, the anti-inflammatory activity of imperatorin (**34**), psoralen (**13**), and seselin (**14**) was investigated using an ear edema model in mice (Garcia-Argaez et al. 2000). The anti-inflammatory potential of the sesquiterpenyl coumarin, umbelliprenin (**16**), was demonstrated in C57/BL6 mice (Khaghanzadeh et al. 2017), and its effect on the acute, chronic, and neuropathic pain was shown in albino mice (Hashemzaei et al. 2015).

Bergapten (**27**) possesses anti-inflammatory property, and this was supported by an *in vivo* study conducted using a zebrafish model (Yang et al. 2018b). Coumarin **27** could effectively inhibit the tail cutting-induced production of reactive oxygen species (ROS) and NO in zebrafish larvae, suggesting **27** could be a potential candidate for inflammation therapy. Zhou et al. (2017) provided further evidence for anti-inflammatory potential of **27** through *in vivo* study; this compound prevented LPS-induced inflammation in RAW264.7 cells through suppressing Janus kinase/signal transducers and activators of transcription (JAK/STAT) activation and ROS production as well as decreased mortality rate of mice after LPS challenge. Two other furanocoumarins, xanthotoxol (**33**) and imperatorin (**34**), were found to possess anti-inflammatory property in an *in vivo* study involving rats (Kumatia et al. 2017; Zhang et al. 2017b).

35.4.8 Anti-obesity

Obesity is a medical condition where excess body fat is accumulated to an extent that it may have a negative effect on health. Obesity has become a global health problem, mainly caused by eating too much (especially fat and sugar) and moving too little (no exercise or physical activities). Some plant-based diets can help treat and manage obesity, and many of those diets contain anti-obesity coumarins. For example, the anti-obesity potential of the geranyloxy furanocoumarin, bergamottin (**29**), a major a component of grapefruit juice, was demonstrated *in vivo* in a diet-induced obese mice model (Ko et al. 2018). The regulatory role of coumarin **29** in lipolysis is well known. Bergamottin (**29**) administration in a high-fat diet-induced obese mice model could reduce the weight and sizes of white adipose tissue as well as the weight gain

of mice fed with high-fat diet. The expressions of the brown adipocyte marker genes, uncoupling protein 1 (UCP1) and peroxisome proliferator-activated receptor-γ coactivator 1 (PGC1) alpha, were higher in the bergamottin-treated high-fat diet mice than that in the high-fat-diet-induced obese mice. Antihyperlipidemic activity of umbelliferone (**2**) and xanthotoxin (**32**) was shown in a mouse model (Iyer and Patil 2014); a considerable reduction in total cholesterol, triglycerides, and low-density lipoproteins and a significant increase in high-density lipoprotein were observed after the treatment with **2** and **32**.

35.4.9 Antipsychotic and Antidepressant

Simply, psychosis can be defined as an impaired relationship with reality, often manifested by serious mental disorders. People who are experiencing psychosis may have either hallucinations or delusions. Hallucinations are sensory experiences that take place within the absence of an actual stimulus. Pandy and Vijeepallam (2017) reported the antipsychotic-like activity of scopoletin (**9**) in a mice model, assessing apomorphine-induced climbing behavior and methamphetamine-induced stereotypy, and concluded that **9** could alleviate positive symptoms of schizophrenia only at a specific dose. Cao et al. (2017) used prenatally stressed offspring rats to investigate the potential antidepressant-like effects of imperatorin (**34**). After 4 weeks of treatment, coumarin **34** exhibited antidepressant-like effects and increased 5-hydroxytryptanin (serotonin) concentration in male prenatally stressed offspring, providing some evidence for the therapeutic use of **34** in prevention of depression-like behavior in adolescents.

35.4.10 Antitumour and Anticancer

Auraptene (**11**), a geranylated simple coumarin, widely occurs in the edible fruits of the genus *Citrus* (Murakami et al. 1997). This compound possesses antitumour property as evident from an *in vivo* animal experimentation; topical application of auraptene (at 160 nmol) could reduce tumor incidence and the numbers of tumors per mouse by 27% ($P < 0.01$) and 23% ($P < 0.05$), respectively (Murakami et al. 1997). Coumarin **11** possesses anticancer potential as evident from numerous *in vitro* studies, but its effectiveness in suppressing renal carcinoma progression in a mouse model was reported by Jang et al. (2015).

Administration of angelicin (**12**), an angular furanocoumarin, to mice bearing liver tumor xenografts inhibited tumor growth, without producing noticeable secondary side effects, suggesting its potential as a novel therapeutic agent for the treatment of patients with liver cancer (Wang et al. 2017a). Angelicin (**12**) and its isomer, psoralen (**13**), were equally effective in treating osteosarcoma in nude rats, in both low- and high-dose groups (Lu et al. 2014). They could induce apoptosis or necrosis of osteosarcoma.

Anticancer property of umbelliferone (**2**) was shown in a chemically induced renal carcinoma model in rats (Anwar et al. 2015). Kim et al. (2018b) reported that esculetin (**8**) could suppress tumor growth and metastasis by targeting Axin2/E-cadherin axis in colorectal cancer *in vivo* using an HCT116-implanted orthotopic mouse model. Zhu et al. (2018a) showed the inhibitory effect of compound **8** on the growth of lung cancer xenograft, possibly mediated by downregulating the expression of the oncogene C-myc, Cyclin D1 (a protein required for progression through the G1 phase of the cell cycle) and NF-κB. Esculetin (**8**) was shown to possess cancer chemopreventive potential *in vivo* using the 7,12-dimethylbenz(a)anthracene-induced hamster buccal pouch carcinogenesis model (Selvasundaram et al. 2018). A supplementation with coumarin **8** could modulate C-myc-induced energy metabolism and attenuate neoplastic changes in rats challenged with the procarcinogen 1,2-dimethylhydrazine (Sharma et al. 2017). Esculetin 3-methyl ether, also known as scopoletin (**9**), inhibited human tumor vascularization in xenograft models (Tabana et al. 2016); it (100 and 200 mg/kg) strongly inhibited (59.72 and 89.4%, respectively) vascularization in matrigel plugs implanted in nude mice.

Antitumour activity of the prenylated coumarin, osthol (**10**), is well documented, and its effectiveness in inhibiting tumour growth in hepatocellular carcinoma through the induction of apoptosis and enhancement of antitumor immune responses in mice was established (Yao et al. 2018b). Furthermore, the role of angiogenesis in the proliferation, invasion, and metastasis of tumour cells in hepatocellular carcinoma was evident in several animal studies. Yao et al. (2018b), using an orthotopic mouse model of hepatocellular carcinoma, proved that **10** could inhibit angiogenesis, which might be one of the mechanisms underlying its antihepatocellular carcinoma activity, possibly mediated by the NF-B/VEGF (vascular endothelial growth factor) signaling pathway. This coumarin was also shown to increase the sensitivity of nasopharyngeal carcinoma in nude mice to radiotherapy by a possible mechanism of downregulation of expressions of vascular endothelial growth factor and hypoxia-inducible factor-l alpha (HIF-1α) and inhibition of angiogenesis (Chen et al. 2018b). Osthol (**10**) is able to inhibit bone metastasis of breast cancer, as demonstrated by Wu et al. (2017) in a mice model of breast cancer osseous metastasis. Mice treated with **10** developed considerably less bone metastases and displayed decreased tumor burden; it inhibited breast cancer cell growth, migration, and invasion and induced apoptosis of breast cancer cells. Furthermore, osthol (**10**) regulated OPG (osteoprotegerin)/RANKL (receptor activator of NF-κB ligand) signals in the interactions between bone cells (osteoblasts and osteoclasts) and cancer cells and inhibited TGFβ (transforming growth factor β)/Smads signaling in breast cancer metastasis to bone in MDA-231BO cells. Note that Smad transcription factors lie at the core of one of the most versatile cytokine signaling pathways in metazoan biology – the TGFβ pathway.

Umbelliprenin (**16**), a farnesyloxy coumarin constituent of *Citrus* fruits, was shown to possess antineoplastic and immunostimulatory effects in 4 T1 mammary tumour-bearing mice, when 12.5 mg/mL of 16 was intraperitoneally administrated to healthy and tumour-bearing mice for 18 days (Rashidi et al. 2018). Coumarin **16** decreased tumour size, augmented serum interferon gamma, and reduced IL-4,

suggesting that this compound could inhibit tumor growth, angiogenesis, metastasis, and inflammation and potentiate an antitumor immune response *in vivo*. The same group also reported its effectiveness against colorectal cancer, developed in mice by intradermal injection of CT26 cell line (Alizadeh et al. 2018). Earlier, Khaghanzadeh et al. (2014) provided *in vivo* evidence in support of the cytotoxic effect of **16** on predominance of the responses of the T helper cells, Th1 and Th2 responses, in Lewis lung cancer mouse model. The cancer chemopreventive activity of **16** *in vivo* by using a two-stage carcinogenesis assay of mouse skin tumours induced by peroxynitrite as an initiator and 12-*O*-tetradecanoylphorbol-13-acetate as a promoter was reported by Iranshahi et al. (2009). This coumarin reduced the number of tumours, and the pattern of tumour promotion was slower in mice treated with **16**.

It is well known that many naturally occurring coumarins, especially *Citrus* coumarins, exert anticarcinogenic effect partly by inducing carcinogen-detoxifying enzymes glutathione S-transferase (GST) and/or NAD(P)H quinone oxidoreductase (NQO1). A comparison of such coumarins, auraptene (**11**), imperatorin (**34**), and limettin (**25**), on carcinogen-detoxifying enzymes in Nrf2 knockout mice was carried out by Prince et al. (2009). It was observed that coumarins **11** and **34** induced murine liver cytosolic GST activities via the Nrf2/ARE mechanism.

Bergapten (**27**) possesses anticancer potential as evident from various animal studies (Pattanayak et al. 2018). It was shown in an *in vivo* study that **27** could ameliorate the cancer-induced alterations in body weight and liver weight and significant restoration of the changes in mRNA and protein expressions of liver X receptor (LXR) (α, β). It could reduce the lipid droplets level in liver cancer cells. Pattanayak et al. (2018) provided evidence and validated the critical role of **27** in maintaining the lipid homeostasis justifying its anticancer potential against NDEA-induced hepatocellular carcinoma. The antitumour potential of umbelliferone (**2**) and xanthotoxin (**32**) was demonstrated in an *in vivo* murine mouse melanoma model (Iyer and Patil 2014).

The antitumour potential of another *Citrus* furanocoumarin, bergamottin (**29**), was shown *in vivo* using a nude mouse model (Wu et al. 2016); an intraperitoneal injection of **29** induced dose-dependent as well as time-dependent cytotoxic effects and inhibition of colony formation in the A549 cancer cells. It also suppressed cancer cell invasion and migration. The doses of 25, 50, and 100 mg/kg bergamottin (**29**) injection reduced the tumour weight from 1.61 g in the phosphate-buffered saline (PBS)-treated group (control) to 1.21, 0.42, and 0.15 g in the bergamottin-treated groups, respectively. It was established that **29** could inhibit lung cancer cell growth in both a cell model and a xenograft mouse model by inducing apoptosis, mitochondrial membrane potential loss, and G2/M cell cycle arrest as well as inhibiting cell migration and invasion.

Anti-skin tumour potential of another furanocoumarin, isopimpinellin (**31**), was reported by Kleiner et al. (2002), who studied the effect of an oral administration of **31** on skin tumour initiation by topically applied benzo[1]pyrene and 7.12-dimethylbenz [a]anthracene. The anticancer potential of imperatorin (**34**), isoimperatorin (**38**), oxypeucedanin (**39**), and oxypeucedanin hydrate (**40**) was established *in vivo* using a melanin possessing hairless mice implanted with B16F10 cells under UV radiation

(Kimura et al. 2013). UV radiation together with these coumarins could reduce tumor growth and final tumor weight in B16F10-bearing mice.

Dong et al. (2018) reported the antileukemic potential of the dihydrofuranocoumarin, marmesin (**41**). In addition to *in vitro* studies, they showed that marmesin (**41**) could prevent the tumour growth at a dose of 30 mg/kg *in vivo* using a rat model.

35.4.11 Asthma and Anti-allergic

The protective effects of angelicin (**12**) on allergic asthma induced by ovalbumin (abbreviated OVA) was studied in mice by Wei et al. (2016); this angular furanocoumarin inhibited inflammatory cells' infiltration into the lungs, attenuated ovalbumin-induced lung injury, and inhibited airway hyperresponsiveness and NF-κB activation. Note that OVA is the main protein found in egg white, making up approximately 55% of the total protein, and it displays sequence and three-dimensional homology to the serpin superfamily. However, unlike most serpins, it is not a serine protease inhibitor.

Potential anti-asthmatic effect of esculetin (**8**) and its plausible mechanism were evaluated by Long (2016) in a mouse model of allergic asthma, which was developed with the sensitization and challenge of OVA. Coumarin **8** could inhibit OVA-induced eosinophil count and recover IL-4, IL-5, IL-13, and IL-17A levels in bronchoalveolar lavage fluid. It was suggested that **8** might effectively ameliorate the progression of asthma and have therapeutic potential in patients with allergic asthma.

The anti-allergic effects of osthol (**10**) in asthmatic mice and its immunomodulatory actions on dendritic cells and T cells were studied by Chiang et al. (2017); an oral administration of 10 to BALB/c mice after OVA sensitization ameliorated all of the cardinal features of T helper 2 (Th2)-mediated allergic asthma. This study provided evidence for the potential therapeutic application of **10** to treat allergic asthma. Anti-asthmatic property of **10** in an OVA-induced asthma model in murine was also reported in the same year (Wang et al. 2017c). Osthol treatment reduced the OVA-induced increase in serum immunoglobulin E (IgE) and inflammatory cytokines, IL-4, IL-5, and IL-13, in bronchoalveolar lavage fluid, and decreased the recruitment of inflammatory cells in lavage fluid and the lung. It also attenuated goblet cell hyperplasia and mucus overproduction in lung tissue. All these findings supported potential pharmaceutical applications of osthol (**10**) in the treatment for asthma and other airway inflammation disorders.

Scoparone (**22**) was reported to inhibit inflammation by lowering the contents of IL-5 in serum and bronchial alveolar lavage fluid and polylactic acid in pulmonary tissues in an asthmatic guinea pig model (Fan et al. 2006). Allergic rhinitis is inflammation of the inside of the nose caused by an allergen, such as pollen, dust, mold, or flakes of skin from certain animals, and a study conducted by Cheng et al. (2013) demonstrated the effectiveness of **22** against this condition in a rat model. Scoparone was highly effective in treating allergic rhinitis by regulating the expression of Th1/Th2 cytokines and IgE.

The anti-allergic property, manifested by antihistaminic effect, of several dietary coumarins, e.g., bergapten (**27**), byakangelicol (**37**), imperatorin (**34**), isoimperatorin (**38**), isopimpinellin (**31**) oxypeucedanin (**39**), oxypeucedanin hydrate (**40**), phellopterin (**35**), scopoletin (**9**), xanthotoxin (**32**), and xanthotoxol (**33**), was reported by Li and Wu (2017). Among these compounds, **27** and **40** were the most effective coumarins. Xian et al. (2018) evaluated the inhibitory effect of imperatorin **34** on airway remodeling model of asthmatic mice and established its possible mechanisms. In an earlier study conducted by Li et al. (2016b), **34** was shown to inhibit allergic airway inflammatory reaction and mucin secretion in OVA-induced asthmatic rats *via* suppressing IgE, histamine, inflammatory cells, and cytokines and also through downregulating the protein coding gene MUC5AC (mucin 5 AC, oligomeric mucus/gel forming). A similar effect was observed with **38** (Wijerathne et al. 2017); this coumarin could attenuate airway inflammation and mucus hypersecretion in an OVA-induced murine model of asthma. Coumarin **38** inhibited OVA-induced inflammatory cell infiltration and mucus production in the respiratory tract, and the pretreatment with **38** suppressed the activation of p38 mitogen-activated protein kinase (p38 MAPK), extracellular-signal-regulated kinases1/2 (ERK1/1) and NF-κB.

35.4.12 Cardioprotection

Cardioprotection comprises several regimens that preserve function and viability of cardiac muscle cell tissue subjected to ischemic insult or re-oxygenation. Umbelliferone (**2**) could provide cardioprotection in coronary artery ligation-induced myocardial ischemia in rats through inflammation and apoptosis pathways (Gan et al. 2018); this effect was also demonstrated by Luo et al. (2018) in a rat model. Jagadeesh et al. (2016) reported the protective effects of **2** in dyslipidemia (abnormal amounts of lipids in blood) and cardiac hypertrophy (abnormal enlargement or thickening of the heart muscle) in isoproterenol-induced myocardial infarction in rats.

Esculetin (**8**) and telmisartan combination could alleviate the pathological features of type 2 diabetic cardiomyopathy including metabolic perturbations, morphometric alterations, altered vascular reactivity, increased Keap1 and fibronectin expression more effectively than their respective monotherapy (Kadakol et al. 2017a). Earlier, the cardioprotective effect of **8** on myocardial ischemia/reperfusion damage in rats was demonstrated (Tao et al. 2016), and potential mechanism was also studied. Coumarin **8** could attenuate myocardial ischemia/reperfusion-induced injury in rats by inhibiting the receptor-interacting protein 140 (RIP140)/nuclear factor kappa B (NF-κB) inflammatory pathway. Another simple coumarin, scoparone (**22**), also known as 3,4-dimethoxycoumarin, was found to possess cardioprotective property in an *in vivo* rat model of ischemia-reperfusion injury and an *in vitro* primary cultured cardiac myocyte model of oxygen-glucose deprivation/re-oxygenation (Wan et al. 2018). The treatment with **22** increased cell viability, superoxide dismutase levels, and B cell lymphoma 2 protein expression

and decreased lactate dehydrogenase release, malondialdehyde production, creatine kinase levels, reactive oxygen species concentration, cell apoptotic rate, myocardial infarct area, and Bcl-2-associated X protein, and caspase-3 and cytochrome c protein expression. Effect of this compound against cardiac fibrosis was reported by Fu et al. (2018), where the possible remedial effect of **22** on angiotensin II-induced extracellular matrix remodeling and its possible mechanism in cardiac fibroblasts was established.

In a recent study, osthol (**10**) was implicated to cardiac protection *in vivo*; this coumarin was observed to decrease collagen I/III contents and their ratio in TGF-β 1-overexpressed mouse cardiac fibroblasts through regulating the TGF-β/Smad signaling pathway (Liu et al. 2018c). It was suggested that **10** might play a beneficial part in the prevention and treatment of myocardial fibrosis. Note that cardiac fibrosis is an abnormal thickening of the heart valves due to inappropriate proliferation of cardiac fibroblasts but more commonly referred to the excess deposition of extracellular matrix in the cardiac muscle. This coumarin was also reported to attenuate right ventricular remodeling via decreased myocardial apoptosis and inflammation in monocrotaline-induced rats (Li et al. 2018a) and to attenuate myocardial ischemia/reperfusion injury in rats by inhibiting apoptosis and inflammation through attenuating pro-inflammatory cytokines, including TNFα and IL-6 and IL-1β, providing evidence for its cardioprotective effect (Wu et al. 2018a).

Another coumarin, imperatorin (**34**), also possesses cardioprotective property. In a study conducted by Zhang et al. (2012), it was observed that coumarin **34** could prevent cardiac hypertrophy and the transition to heart failure via nitric oxide (NO)-dependent mechanisms in mice. Marmesin (**41**), a dihydrofuranocoumarin, was shown to provide protection in myocardial infraction in animal model (Krushna et al. 2017); the findings were also supported by molecular docking studies.

35.4.13 Gastroprotection

Li et al. (2017c) reported the gastroprotective property of esculin (**18**) in a mouse model of ethanol-induced gastric lesion. Pretreatment with this coumarin could reduce macroscopic and histopathological damage and gastric lesion index in a dose-dependent fashion. It could also reduce NO production, inducible NO synthase (iNOS) levels, and NF-κB p65 protein expression in gastric tissues after ethanol challenge and suppress the increased expression of tumour TNF-α and IL-6 in ethanol-treated mice. The underlying mechanism of gastroprotection offered by **18** was assumed to be linked to inhibition of NF-κB activation, which subsequently reduces expression of iNOS, TNF-α, and IL-6. Scoparone (**22**) could offer gastroprotection through its anti-ulcerogenic property as observed on HCl/ethanol-induced gastritis in rats (Choi et al. 2012). In a similar animal model, scopoletin (**9**) and scopolin (**23**) showed anti-ulcerogenic activity at a dose of 50 mg/kg (Awad et al. 2015).

Sekiguchi et al. (2012) reported the gastroprotective effect of auraptene (**11**), a key component of *Citrus* fruit peels; it could suppress *Helicobacter pylori* adhesion

via expression of CD74 (an integral transmembrane molecule), which has been identified as a new receptor for *H. pylori* urease. *Helicobacter pylori* is a major human pathogen that plays central roles in chronic gastritis and gastric cancer. This coumarin inhibited *Helicobacter pylori*-induced expression and/or production of CD74, macrophage migration inhibitory factor, IL-1β, and TNF-α in gastric mucosa, together with serum macrophage inhibitory protein-2. Thus, auraptene (**11**) could be a promising dietary coumarin for reducing the risk of *H. pylori*-induced gastritis and carcinogenesis.

35.4.14 Hepatoprotection

Hepatoprotection or antihepatotoxicity is the ability of a substance to prevent damage to the liver cells and thus the liver. The protection of the liver cells against toxic substances, e.g., drugs, lipid peroxidation, and free radical injury, may decrease inflammation, improve liver blood flow, and ultimately help in reduction of ascites and blood pressure. Hepatoprotecive property of esculin (**18**), a glucoside of the coumarin esculetin (**8**), was evaluated in a mice model (Liu et al. 2018a). Its hepatoprotection is generally mediated through its antioxidant and anti-inflammatory effects. Liu et al. (2018a) showed that this coumarin glucoside (**18**) could reduce the pathological symptoms of induced acute liver injury in mice, as well as serum AST (aspartate aminotransferase) and ALT (alanine aminotransferase) levels. While lipopolysaccharide (LPS)/D-Gal-induced liver myeloperoxidase (MPO) activity and malondialdehyde (MDA) content were suppressed by **18**, LPS/D-Gal-induced liver TNF-α and IL-1β production were attenuated by this compound. Furthermore, **18** inhibited NF-κB activation as well as increased nuclear factor E2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) expression.

Hepatoprotective potential of the simple coumarin, umbelliferone (**2**), was evaluated in diabetic mice, and it was suggested that the effect was mediated possibly through inhibition of HMGB1 (*high mobility group box 1* protein)-induced inflammatory response and activation of Nrf2-induced antioxidant property (Yin et al. 2018). Possible involvement of Nrf2 and PPAR-γ (peroxisome proliferator-activated receptor gamma) upregulation in the protective effect of **2** against cyclophosphamide-induced hepatotoxicity was demonstrated by Mahmoud et al. (2017). Hepatoprotective effect of **2** and **8** was demonstrated in *N*-nitrosodiethylamine-induced hepatotoxicity in rats (Subramaniam and Ellis 2016). Earlier, Kim et al. (2014) reported that dietary intake of coumarin **2** could attenuate alcohol-induced fatty liver *via* regulation of PPAR-α and SREBP (*sterol regulatory element-binding proteins*)-1c in rats; this compound could attenuate alcoholic steatosis through downregulation of SREBP-1c-mediated lipogenesis and upregulation of PPAR-α-mediated fatty acid oxidation. While hepatoprotective property of **2** and its associated mechanisms of action were also reported by Mohamed et al. (2014), this effect was demonstrated for **8** on ethanol-induced liver injury in human HepG2 cells and in a mouse model (Lee et al. 2018).

Esculetin (**8**) prevented ethanol-induced liver injury by reducing glutathione depletion, malondialdehyde production, and the lipid contents in the liver. Moreover,

this compound lowered the levels of serum ALT, serum AST, and the content of serum lipids. Hepatoprotective potential of compound **8** was also reported by Pandey et al. (2017) in a high-fat-diet-induced non-alcoholic fatty liver disease mouse model; esculetin (**8**) was effective in improving liver fibrosis. A similar work was carried out by Choi et al. (2016), and they reported that coumarin **8** could protect against development of non-alcoholic fatty liver in diabetes *via* regulation of lipids, glucose, and inflammation. Hepatoprotective effect of scopoletin (**9**) was shown using a high-fat-diet-fed mice model, as part of the investigation looking at the effects of this compound on non-alcoholic fatty liver in obese mice (Ham et al. 2016). It was observed that at the doses of 0.01 and 0.05% w/w for 16 weeks, **9** could reduce body weight, visceral fat, serum levels of leptin, lipid, TNF-α, IL-6, interferon (IFN)-γ and MCP-1 (monocyte chemoattractant protein-1), insulin resistance, and hepatic lipid accumulation. It was postulated that this coumarin could possibly treat high-fat-induced hepatic dysfunction via regulation of lipid metabolic and inflammatory genes. The hepatoprotective effect of scoparone (**22**), which is actually scopoletin methyl ether, was demonstrated by Liu and Zhao (2017). Earlier, Kang et al. (2013) showed that **22** could attenuate D-galactosamine/LPS-induced fulminant hepatic failure through inhibition of toll-like receptor 4 signaling in mice.

Isofraxidin (**19**) is thought to play a protective role against lipid metabolism disorder induced by a high-fat diet *via* inhibition of lipid production and inflammation in the liver. Li et al. (2017a) demonstrated that the treatment with **19** could inhibit the high-fat-diet-induced elevation in body weight, liver weight, lipid metabolism, and hepatic injury in mice. The anti-inflammatory activity in isofraxidin-treated hepatic tissue correlated with downregulation of TLR4 (toll-like receptor 4) expression and NF-κB transcription. Scopolin (**23**), which is the glucoside of scopoletin (**9**), could exhibit beneficial effect on high-fat-diet-induced hepatic steatosis in mice (Yoo et al. 2017). This coumarin glycoside could attenuate hepatic steatosis through activation of sirtuin 1-mediated signaling cascades, a potent regulator of lipid homeostasis, and increase hepatic sirtuin 1 activity and protein expression.

Potential hepatoprotective effects of osthol (**10**) at a dose of 100 mg/kg (i.p.) was shown by Zhou et al. (2018) using a tamoxifen-induced acute liver injury in mice. Pretreatment with **10** could attenuate tamoxifen-induced liver injury evident from dose-dependent reduction of serum ALT and AST activities and could counteract tamoxifen-induced oxidative stress, evident from marked increase of reduced glutathione and reduction of malondialdehyde and hydrogen peroxide. It was concluded that **10** could prevent tamoxifen-induced liver toxicity by suppressing p38 activation and subsequently reduce tamoxifen-induced oxidative damage. Coumarin **10** could ameliorate the hepatic lipid metabolism and inflammatory response in nonalcoholic steatohepatitic rats, and the effects might be mediated by the activation of hepatic peroxisome proliferator-activated receptor alpha/gamma (PPAR-α/ PPAR-γ) (Zhao et al. 2018c). The hepatoprotective potential of **10** and its possible mechanisms of action were studied in several animal models (Cai et al. 2018; Zhao et al. 2018c).

Hepatoprotection of auraptene (**11**), a coumarin found in the peels of *Citrus* fruits such as grapefruits, was evaluated against thioacetamide-induced hepatic fibrosis in

mice (Gao et al. 2018). They showed that **11** could protect against liver injury induced by thioacetamide in mice and maintain the homeostasis of bile acids *via* the regulation of FXR (farnesoid X receptor) target genes, reducing collagen content in the liver and inhibiting the activation of hepatic stellate cells by downregulating the expression of TGF-β1 and α-SMA (smooth muscle actin). Hepatoprotection-related anti-inflammatory effects of **11** were mediated by reducing the expression of NF-κB, TNF-α, and IL-1β. Earlier, the effect of dietary auraptene (**11**) on hepatic lipid metabolism both *in vitro* and *in vivo* was studied. It was shown that this coumarin could normalize lipid abnormalities in HepG2 hepatocytes (Nagao et al. 2010), and administration of **11** alleviated obesity and hepatic triglyceride accumulation in part through lipolysis enhancement in the livers of obese Otsuka Long-Evans Tokushima fatty rats.

Hepatoprotective effect of bergamottin (**29**) was demonstrated in a paracetamol-induced hepatotoxicity model in rats (Baleni et al. 2015). Hepatoprotection mediated through carcinogen-detoxifying effect of furanocoumarins, imperatorin (**34**) and isopimpinellin (**31**), and **11**, was demonstrated by Prince et al. (2009). It was observed that coumarin **31** could increase liver cytosolic NAD(P)H quinone oxidoreductase (NQO1) activities, and its effect was not attenuated in Nrf2(−/−) mice, suggesting that the coumarins **11** and **34** could induce murine liver cytosolic glutathione S-transferase (GST) activities via the Nrf2/ARE mechanism. It was also noted that despite structural similarities, **31** could not activate HepG2-ARE-GFP (green fluorescent protein), and in the Nrf2 knockout mouse, compound **31** induced GST and NQO1 via additional mechanisms.

35.4.15 Immunomodulation

Bergapten (**27**) has immunomodulatory effects, which was shown to be responsible for its ability to attenuate D-galactose-induced aging in mice (Xie et al. 2018). Bergapten-treated group could reverse body weight and spleen index in aging progress, promote T cell proliferation, and upregulate IFN-γ and IL-4 in aging mice. Additionally, this compound could enhance T helper cell (Th) and cytotoxic T cell (Tc) responses, which seemed to participate in the process of eliminating the virus in an old age.

35.4.16 Lung Injury Protection

Umbelliferone (**2**) attenuated LPS-induced acute lung injury in mice, possibly *via* the anti-inflammatory and anti-apoptotic activities (Luo et al. 2016). Like coumarin **2**, esculetin (**8**) was also shown to possess protective effects in lung injury (Chen et al. 2015). It was shown that esculetin (**8**) could resolve lipopolysaccharide (LPS)-induced acute lung injury via regulation of RhoA/Rho kinase/NF-κB pathways *in vivo* and *in vitro*. It can be noted that the Rho A/Rho kinase pathway plays an

important role in the development of hypoxic pulmonary hypertension, which contributes to the pathogenesis of cardiopulmonary diseases.

Similarly, scopoletin (**9**) could offer protection against cerulein-induced acute pancreatitis and associated lung injury in mice (Leema and Tamizhselvi 2018). The protective effects and potential mechanism of action of isofraxidin (**19**) against LPS-induced acute lung injury in mice were reported by Niu et al. (2015b); the pre-treatment of **19** lowered LPS-induced mortality and lung wet-to-dry weight ratio and reduced the levels of TNF-α, IL-6, and prostaglandin E-2 (PGE-2) in serum and bronchoalveolar lavage fluid. The protective effect of **19** appears to be mediated from the inhibition of COX-2 protein expression in the lung, which regulates the production of PGE-2. Another simple coumarin, scoparone (**22**), was found to project LPS-induced acute lung injury in mice *via* suppression of TLR4-mediated NF-κB signaling pathways (Niu et al. 2014).

Osthol (**10**) is able to protect against acute lung injury. In a recent study, it was found that this coumarin could protect against acute lung injury by suppressing NF-κB-dependent inflammation in mouse (Jin et al. 2018). Coumarin **10** treatment inhibited the LPS-induced inflammatory response in mouse peritoneal macrophages through blocking the nuclear translocation of NF-κB. Preventative effect of imperatorin (**34**) on acute lung injury induced by LPS in mice was reported by Sun et al. (2012). BALB/c mice were pretreated with **34** 1 h prior to LPS challenge. The levels of TNF-α, IL-1, and IL-6 in the bronchoalveolar lavage fluid were reduced significantly, and the level of IL-10 was upregulated 10 h after the imperatorin treatment. Pretreatment with **34** decreased lung wet-to-dry ration, the number of inflammatory cells, and myeloperoxidase activities.

35.4.17 Nephroprotection

Nephroprotection by esculin (**18**) in a diabetic animal model, mediated through its established antioxidant and anti-inflammatory routes, was demonstrated by Wang et al. (2015a). This compound could improve dyslipidemia, inflammation responses, and renal damage in streptozotocin-induced diabetic rats. Nephroprotective effect of this compound was also reported by Kang et al. (2014) on streptozotocin-induced diabetic renal damage in mice. Naaz et al. (2014) demonstrated the protective properties of **18** against pro-oxidant aflatoxin B-1-induced nephrotoxicity in mice, which was implicated to its antioxidant and free radical-scavenging activities. Nephroprotection was also shown to be offered by umbelliferone (**2**) in methotrexate-induced renal injury in adult male albino rats (Hassanein et al. 2018). It was concluded that the nephroprotective effect of **2** was through modulation of oxidative stress and inflammation and apoptosis with enhancement of its cytotoxicity. Like umbelliferone (**2**), esculetin (**8**) could also offer nephroprotection in type 2 diabetic rats (Kadakol et al. 2017b).

Osthol (**10**), well known for its inhibitory effects on inflammation, oxidative stress, fibrosis, and tumor progression, could offer nephroprotection by ameliorating renal fibrosis, which is a common pathway of virtually all progressive kidney

diseases, in mice (Zhang et al. 2018b). Coumarin **10** could hinder renal fibrosis in unilateral ureteral obstruction mouse mainly through inhibition of fibroblast activation and epithelial-mesenchymal transition. Earlier, the nephroprotective potential of osthol **10** in a chronic kidney failure rat model was established by. They showed that this compound could protect against inflammation in a rat model of chronic kidney failure *via* suppression of NF-κB and transforming growth factor-beta 1 and activation of phosphoinositide 3-kinase/protein kinase B/nuclear factor (erythroid-derived 2)-like 2 signaling. Osthol (**10**) treatment reversed chronic kidney failure-associated changes in serum creatinine, calcium, phosphorus, and blood urea nitrogen levels in chronic kidney failure rats. It inhibited chronic kidney failure-induced TNF-α, IL-8, and IL-6 expressions and suppressed NF-κB protein expression. Furthermore, it attenuated the protein expression of transforming growth factor-beta 1 (TGF-β1), reduced monocyte chemoattractant protein-1 activity, and increased the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) ratio. The nephroprotective activity of imperatorin (**34**), mediated by its antihypertensive and antioxidant property, was demonstrated in a rat model (Cao et al. 2013), where **34** showed antihypertensive and antioxidant effects in the renal injury of renovascular hypertensive rats, providing evidence for its potential therapeutic use in the prevention of renal injury related hypertension.

35.4.18 Neuroprotection

Xanthotoxin (**32**) is one of the most common furanocoumarins present in food plants, especially from the family Apiaceae (Table 1), and it has been implicated to many bioactivities. In a recent study (Skalicka-Wozniak et al. 2018), the effect of **32** was investigated on the scopolamine-induced cognitive deficits in male Swiss mice using the passive avoidance test. Either single (2.5 and 5 mg/kg) or repeated (1 mg/kg) administration of this coumarin could increase index of latency in both acquisition and consolidation of memory processes linking to some procognitive effects. An acute (2.5 mg/kg) and subchronic (1 mg/kg) administration of **32** could prevent memory impairment induced by the injection of scopolamine (1 mg/kg). Based on these findings, it was suggested that **32** might possess neuroprotective effect in scopolamine-induced cognitive impairment connected to cholinergic neurotransmission and oxidative stress in the brain structures. Earlier, the same group showed that **32**, its isomer bergapten (**27**), and the simple coumarin umbelliferone (**2**), which are known as CYP2A6 inhibitors, could prolong the antidepressive and procognitive effects of nicotine as demonstrated by the forced swimming test (animal models of depression) and passive avoidance test (memory and learning paradigm model) (Budzynska et al. 2016). The same group also reported the anticonvulsant potential of **27**, **32**, imperatorin (**34**), and oxypeucedanin (**39**), using mouse model (Luszczki et al. 2010; Skalicka-Wozniak et al. 2014). Li et al. (2017b) reported estrogen-like neuroprotection of angelicin (**12**) in an animal model of spinal cord injury; intraperitoneal injections (5 and 10 mg/kg per day for 2 weeks) enhanced the hind limb locomotor functions of mice with spinal cord injury.

Antidepressant effect, mediated though associating signaling with neuroinflammation and neurogenesis, of the simple coumarins esculetin (**8**) and its glucoside (esculin, **18**) in a mouse model of chronic stress-induced depression was reported (Kim et al. 2018a). Umbelliferone (**2**) was shown to reverse depression-like behaviour in chronic unpredictable mild stress-induced rats (Qin et al. 2017). It was suggested that the neuroprotective effects on chronic unpredictable mild stress-induced model of depression could be associated with the inhibition of neuronal apoptosis. Umbelliferone (**2**) is known to cross blood-brain barrier (Subramaniam and Ellis 2013) and to protect neuronal cells from death. This fact was further reinforced by Wang et al. (2015b); the effects of **2** in a rat model of focal cerebral ischemia induced by middle cerebral artery occlusion/reperfusion were studied. They concluded that the possible neuroprotection was offered by **2** against focal cerebral ischemic partly through the inhibition of TXNIP (thioredoxin-interacting protein)/NLRP3 (NOD-like receptor protein 3) inflammasome and activation of PPAR-γ. Neuroprotective effects of coumarins **2** and **8** were reported using a mouse model of Parkinson's disease, where 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity was utilized (Subramaniam and Ellis 2013).

Esculetin (**8**) is a potent antioxidant and anti-inflammatory compound, and it has been shown to possess neuroprotective potentials in various animal models of neurodegeneration (Lin et al. 2017), one of those is the study by Lin et al. (2017), where they elucidated the neuroprotective potential of **8** against streptozotocin-induced experimental dementia of Alzheimer's type in rats. Zhu et al. (2016) provided evidence from *in vivo* experimentation using a rat model to support that neuroprotection of **8** could be attributed to the upregulations of brain-derived neurotrophic factor (BDNF) and phosphorylated tyrosine kinase B (p-TrkB) protein expression in hippocampus. The antidepressant-like effects might be related to the inhibition of NF-κB pathway and the activation of BDNF/TrkB signaling. Dietary antioxidants, like **8**, play an important role in the aging process by relieving oxidative damage, a likely cause of age-associated brain dysfunctions (Martin-Aragon et al. 2016). Esculetin treatment could attenuate the increased immobility time and enhance the diminished climbing time in the forced swim task elicited by acute restraint stress in the 11- and 17-month-old mice versus their counterpart controls (Martin-Aragon et al. 2016). It could also partially reverse the acute restraint stress generated impairment of contextual memory in the step-through passive avoidance both in mature adults. It was postulated that **8** could be useful in preventing the acute restraint stress-induced oxidative stress mostly in mature adult mice by restoring antioxidant enzyme activities.

Scopoletin (**9**) and scopolin (**23**) at the doses of 2.5, 5, 10, and 15 mg/kg, p.o., were evaluated for memory-enhancing activity against scopolamine-induced amnesia using elevated plus maze and step-down paradigms, and the effect on acetylcholinesterase activity in mice brain (Malik et al. 2016). Both coumarins, **9** and **23**, dose-dependently attenuated the scopolamine-induced amnesic effect. In addition, compounds **9** and **23** at the doses of 10 and 15 mg/kg showed activity comparable to that of donepezil, and they also exhibited significant cetylcholinesterase inhibitory activity.

Osthol (**10**), sometimes also spelt as osthole, is a prenylated simple coumarin and could be effective against neurodegenerative diseases, e.g., Alzheimer's disease. Yao et al. (2019) have recently investigated the possible role of **10** in decreasing hyperphosphorylated tau protein in Alzheimer's disease and studied the underlying mechanism in a mice model. Osthol (**10**) improved learning and memory dysfunction and ameliorated the histology structure of damaged neural cells in hippocampal area. It could decrease tau protein phosphorylation as well as inhibit cells apoptosis. Neuroprotection, offered by **10**, was demonstrated in a recent study, where this compound promoted endogenous neural stem cell proliferation and improved neurological function through Notch signaling pathway in mice acute mechanical brain injury (Yan et al. 2018), establishing its potential in neural restoration. Note that the Notch signaling pathway is a highly conserved cell signaling system present in most multicellular organisms, and mammals possess four different notch receptors, i.e., NOTCH1, NOTCH2, NOTCH3, and NOTCH4. The *notch* receptor is a single-pass transmembrane receptor protein. Coumarin **10** was particularly useful in enhancing the therapeutic efficiency of stem cell transplantation in neuroendoscopy caused traumatic brain injury (Tao et al. 2017), and in stimulating neural stem cells differentiation into neurons in an Alzheimer's disease cell model via upregulation of microRNA-9 and rescued the functional impairment of hippocampal neurons in transgenic mice (Li et al. 2017d).

Several other dietary coumarins, e.g., auraptene (**11**) (Okuyama et al. 2016, 2018), also offer neuroprotection. Using an animal model, it was shown that **11** could suppress inflammatory responses including the hyperactivation of microglia in the ischemic brain and inflamed brain, thereby inhibiting neuronal cell death (Okuyama et al. 2016). Ghanbarabadi et al. (2016) also reported the neuroprotective and memory-enhancing effects of **11** in a rat model of vascular dementia. Xanthotoxol (**33**) could offer neuroprotection through suppression of the inflammatory response in a rat model of focal cerebral ischemia as evident from alleviation of brain edema, inhibition of the neutrophil filtration, and reduction of intercellular adhesion molecule-1 and E-selectin (He et al. 2013). It was concluded that the neuroprotection might be linked to xanthotoxol's ability to attenuate the expression of pro-inflammatory mediators and to inhibit the inflammatory response after cerebral ischemia. Neuroprotective effect of imperatorin (**34**) was shown in a LPS-induced memory loss in mice (Chowdhury et al. 2018). Coumarin **34** could ameliorate LPS-induced memory deficit by mitigating pro-inflammatory cytokines and oxidative stress and modulating brain-derived neurotrophic factor. Imperatorin (**34**) pretreatment reversed LPS-induced behavioral and memory disturbances and decreased oxidative stress and acetylcholinesterase levels. It also reduced TNF-α and IL-6 levels and upregulated brain-derived neurotrophic factor in hippocampus and cortex of the brain.

35.4.19 Osteoporosis and Osteoarthritis

Osteoporosis is a condition that weakens bones, making them fragile and more likely to break. Coumarins from *Citrus aurantifolia* and *C. sinensis*, e.g., xanthotoxin (**32**)

and marmesin (**41**), were assessed for the osteoporosis-preventing property of the crude extracts using an ovariectomized rat model (Shalaby et al. 2011), suggesting the potential of these *Citrus* extracts to be developed as clinically useful anti-osteoporotic agents. Earlier, Dou et al. (2016) exhibited that coumarin **32** could prevent bone loss in ovariectomized mice through the inhibition of NF-κB ligand (RANKL)-induced osteoclastogenesis and also could decrease osteoclast number, prevent bone loss, and restore bone strength in mice. Thus, xanthotoxin (**32**), a common coumarin in *Citrus* fruits, might be a new therapeutic candidate for the treatment of osteoporosis.

Bergapten (**27**), another major component of *Citrus* fruits, was also found to exert similar effects in animal models (Chen et al. 2019). In a recent study, this furanocoumarin was shown to suppress receptor activator of NF-κB ligand-induced osteoclastogenesis and ovariectomy-induced osteoporosis *via* suppression of NF-κB and JNK (c-Jun N-terminal kinases) signaling pathways (Chen et al. 2019). Earlier, this compound was shown to inhibit diabetes-related osteoporosis *via* the regulation of the PI3K/AKT, JNK/MAPK, and NF-κB signaling pathways in osteoprotegerin knockout mice and thus to protect trabecular structure and decrease osteoclastogenic differentiation (Li et al. 2016b). This coumarin could also promote bone marrow stromal cell differentiation into osteoblasts *in vitro* and *in vivo* using a mouse model (Xiao et al. 2015). The protective effect of **27** against ovariectomy-induced bone loss was found by distal femur micro-CT scanning, with improvements of bone metabolism parameters such as bone mineral density, trabecular number, and trabecular separation.

Using a similar rat model, Liu et al. (2018b) reported the effect of esculetin (**8**) on bone metabolism. Esculetin-treated group had greater femoral bone mineral density and tibial trabecular bone volume and much smaller trabecular resorption surface, suggesting that coumarin **8** could increase bone mass by upregulating receptor activator of NF-κB ligand (RANKL) expression in osteoblasts and bone marrow stromal cells, and decreasing serum IL-6 concentration, and thus offer its therapeutic effect *via* a decrease in bone resorption.

Angelicin (**12**), also known as isopsoralen, could increase bone strength and trabecular bone microstructure in a sex hormone deficiency-induced osteoporosis model (Ge et al. 2018). This angular furanocoumarin promoted osteoblast differentiation and mineralization, increased calcium nodule levels and alkaline phosphatase (ALP) activity, and upregulated osteoblast markers, including ALP, runt-related transcription factor 2 (RUNX2), and collagen type I alpha 1 chain (COL1A1). It was suggested that **12** could act as an AhR antagonist and promote osteoblast differentiation via the AhR/ER alpha axis. In a similar study involving rats, **12** was shown to inhibit oxidative stress in osteoporosis (Wang et al. 2018a).

Earlier, the same group demonstrated angelicin-mediated suppression of bone marrow adiposity and attenuation of the adipogenic commitment of bone marrow-derived mesenchymal stem cells (Wang et al. 2017b). Yuan et al. (2016) studied the preventative effect of psoralen (**13**) and its isomer (angelicin, **12**) in a mice model and exhibited that these coumarins could ameliorate sex hormone deficiency-induced osteoporosis in female and male mice. After administration of these compounds for 8 weeks, osteoporosis was ameliorated with increasing bone strength and

improving trabecular bone microstructure, suggesting that coumarins **12** and **13** could be good natural compounds for the treatment of osteoporosis in males and females. In a similar study, Zhao et al. (2017) showed that esculin (**18**) could modulate bone metabolism by suppressing receptor activator of NF-κB ligand-induced osteoclastogenesis and transduction signals. They demonstrated that this coumarin could protect against bone mass loss in the ovariectomized and dexamethasone-treated rat osteoporosis model. Psoralen (**13**) has recently been shown to be effective in the treatment of steroid-induced avascular necrosis of femoral head in a rabbit model (Li et al. 2019). Wang et al. (2019c) have demonstrated that **13** could protect and activate chondrocytes, antagonizing the expression of matrix metalloproteinases and interleukins secreted by synovial cells, and effectively attenuates monosodium iodoacetate-induced osteoarthritis. The effect of combination therapy with bone morphogenetic protein (BMP)-2 and **13** on fracture repair in ovariectomized mice was evaluated by Huang et al. (2018), where it was observed that the systematically administered combination therapy could improve bone healing compared with BMP-2 alone. Efficacy of **13** and its derivatives in the treatment of osteoporosis and their possible mechanisms have been demonstrated in various animal models quite extensively (An et al. 2016; Li et al. 2017e).

The effect of scopoletin (**9**)-standardized *Morinda elliptica* leaf extract against osteoarthritis was investigated in *ex vivo* explant culture and preclinical rodent model (Osman et al. 2017). The scopoletin-rich extract dose-dependently reduced serum inflammation biomarkers, increased bone formation biomarkers to near-normal levels in the osteoarthritis-induced rats, and alleviated osteoarthritis progression and articular cartilage structure, by ameliorating cartilage degradation, nitric oxide levels, inflammation, bone/cartilage homeostasis, collagenase/aggrecanase activities, chondrocytes survival, subchondral bone structure, and integrity.

Osthol (**10**) is well known for its osteogenic and anti-osteoporotic properties, as demonstrated in various animal models. Osthol (**10**) could inhibit osteoclastogenesis and thus prevent ovariectomy-induced bone loss in mice at a dose of 10 mg/kg/d for 7 days (Zhao et al. 2018b); the inhibitory effects of **10** on receptor activator of NF-κB ligand (RANKL)-activated osteoclastogenesis were responsible for its bone protective effects in ovariectomized mice. Based on this study, it could be suggested that **10** might be a potential new drug for treating postmenopausal osteoporosis. Zheng et al. (2018) also showed that **10** could be effective in the treatment of osteoporosis in rats and eventually a potential anti-osteoporosis medicine for humans. They investigated the effect of **10** on proliferation and differentiation of osteoblast and observed that the effect of **10** on inhibition of proliferation was relevant with activation of endoplasmic reticulum stress, and activation of Wnt/beta-catenin signaling pathway could be one of the mechanisms how this coumarin could promote osteoblast differentiation. Osthol (**10**) was shown to improve collagen-induced arthritis in a rat model through inhibiting inflammation and cellular stress (Xu et al. 2018). Zhang et al. (2017a) demonstrated the osteopromotive action of coumarin **10** on mouse osteoblastic MC3T3-E1 cells and on mouse femoral fracture repair, and the interaction between osthol-induced osteopromotive effect and cyclic adenosine monophosphate (cAMP) elevating effect. Osthol treatment could promote osteogenesis in osteoblasts by enhancing alkaline

phosphatase activity and mineralization. Oral gavage of osthol (**10**) enhanced fracture repair and increased bone strength. It was concluded that osthol-mediated osteogenesis was related to activation of the cAMP/CREB signaling pathway and downstream osterix expression. Coumarin **10** is able to enhance bone formation and improve fracture healing. Wang et al. (2017d) showed that this compound could promote bone fractures through activation of BMP (bone morphogenetic protein) signaling in chondrocytes and suggested that coumarin **10** could be used as an alternative approach in the orthopedic clinic for the treatment of fracture healing.

Ouyang et al. (2017) reported the potential therapeutic efficacy of isoimperatorin (**38**). In their study using a mouse model for osteoarthritis, it was observed that this compound could ameliorate osteoarthritis by downregulating the mammalian target of rapamycin C1 signaling. Isoimperatorin (**38**) ameliorated osteoarthritis-induced pathological changes by delaying chondrocyte deterioration, activating autophagy, and inhibiting mTORC1.

35.4.20 Periodontitis and Orthodontic Tooth Movement

Periodontitis is inflammation of the gums and supporting structures of the teeth. Periodontitis is caused by certain bacteria (known as periodontal bacteria) and by the local inflammation triggered by those bacteria. Li et al. (2018b) demonstrated the effectiveness of angelicin (**12**) and psoralen (**13**) in the treatment of periodontitis in a mouse model. Orthodontic tooth movement occurs as a result of a force being placed on a tooth and is dependent on efficient remodeling of the bone. Orthodontic treatment is often based on the premise that when force is delivered to a tooth and thereby transmitted to the adjacent investing tissues, certain mechanical, chemical, and cellular events take place within these tissues, resulting in structural alterations and contributing to the movement of that tooth. Wu et al. (2018b) investigated the effects of **13** on relapse after orthodontic tooth movement and its possible molecular mechanism, using a rat model. They found that compound **13** could act as a bone-modifying agent to prevent relapse after orthodontic tooth movement through activating GPR30 and upregulating BMP2 (bone morphogenic protein 2) and BMP4 (bone morphogenic protein 4) expressions.

35.5 Benefits (Human Studies)

The benefits of dietary coumarins for humans have been explored quite considerably. Various reported physiological, bacteriostatic, and antitumor activities of coumarins, mostly based on *in vitro* findings, have put these compounds in the forefront of further backbone derivatization and screening as novel therapeutic agents. For example, it was shown that coumarin (**1**) and umbelliferone (**2**) had antitumor activity against several human tumor cell lines, and coumarins could have potential as inhibitors of cellular proliferation in various carcinoma cell lines (Lacy and O'Kennedy 2004). It was demonstrated that 4-hydroxycoumarin (**21**) and

umbelliferone (**2**) inhibited cell proliferation in a gastric carcinoma cell line (Budzisz et al. 2003). However, subsequently several reports on *in vivo* studies of various coumarins in animal models opened up the opportunities for coumarins to be used in clinical medicine. It had been evaluated for the treatment of various clinical conditions, resulting in the use of a variety of dosing regimens. Recommended doses range from 8 mg for the treatment of venous constriction to 7000 mg/day in anticancer therapies. Auraptene (**11**), isolated from the peels of *Citrus kawachiensis* (Kawachi Bankan) fruits, was shown to contribute to the preservation of cognitive function in a randomized, placebo-controlled, double-blind study in healthy volunteers (Igase et al. 2018). It was observed that coumarin **11** did not improve cognitive function after 24 weeks compared with baseline data, but there was a significant difference in the percentage change in cognitive function between the test and placebo orange juice groups.

Psoralen plus UVA (PUVA) is an effective therapy for mycosis fungoides, which is the skin-limited variant of cutaneous T cell lymphoma (Vieyra-Garcia et al. 2019). In low-burden patients, this therapy reduced or eradicated malignant T cells and induced clonal expansion of CD8+ T cells associated with malignant T cell depletion. Whereas the high-burden patients clinically improved, large numbers of malignant T cells persisted in the skin. Boix-Vilanova et al. (2018) presented a rather rare case of a folliculotropic mycosis fungoides in a 13-year-old boy, which initially presented as a plaque on his face and was treated with local psoralen plus UVA therapy. When the lesions did spread, the treatment was changed to systemic PUVA with good response.

Brass et al. (2018) carried out an observer-blinded randomized controlled pilot study using validated scoring criteria to compare immersion PUVA with narrowband-UVB for the treatment of chronic hand eczema unresponsive to topical steroids and found that both PUVA and narrowband-UVB could reduce the severity of chronic palmar hand eczema. "Turban PUVAsol," which is a modification of topical PUVA used as localized immunotherapy using psoralen (**13**) solution followed by sun exposure, especially for the treatment of alopecia areata, an autoimmune characterized by nonscarring loss of scalp and/or body hairs (Majumdar et al. 2018). This modified psoralen-based therapy was found to be cost-effective and a safer treatment option for alopecia. Mateeva et al. (2018) demonstrated the efficacy of systemic PUVA treatment in Bulgarian patients with mycosis fungoides, especially at the early stage of this disease. It was observed that PUVA therapy alone or in combination with interferon alpha-2a or chemotherapy led to clinical improvement and reduction of the burden of disease in mycosis fungoides in all clinical stages. Earlier, the efficiency of PUVA bath therapy in psoriatic patients was demonstrated by Volnukhin et al. (2006). PUVA bath therapy, which incorporates bergapten (**27**), isopimpinellin (**31**), and xanthotoxin (**32**), was found to be highly effective in psoriatic patients and could be used as an alternative to other forms of PUVA therapies (Volnukhin et al. 2006). In fact, bergapten (**27**) is under investigation in clinical trial NCY00533195 (comparison of UVA1 phototherapy versus photochemotherapy for patients with severe generalized atopic dermatitis). Table 4 summarizes the clinical uses and human benefits of some dietary coumarins.

Table 4 Clinical uses and human benefits of various dietary coumarins

Clinical conditions	Recommended coumarins	References
High protein edema	Coumarin (**1**)	Lacy and O'Kennedy (2004)
Chronic infections	Coumarin (**1**)	Lacy and O'Kennedy (2004)
Malaria	Coumarin derivative from *Toddalia asiatica*	Oketch-Rabah et al. (2000)
Cancer (malignant melanoma, renal cell carcinoma, prostate cancer, leukemia, cervical carcinoma)	Angelicin (**12**), coumarin (**1**), esculetin (**8**), 4-hydroxycoumarin (**21**), psoralen (**13**), and umbelliferone (**2**)	Lacy and O'Kennedy (2004)
Skin disorders (mycosis fungoides, psoriasis, and vitiligo)	Psoralen (**13**)	Lacy and O'Kennedy (2004)
Cognitive decline	Auraptene (**11**)	Igase et al. (2018)
Mycosis fungoides	Psoralen	Mateeva et al. (2018), Vieyra-Garcia et al. (2019)
Psoriasis	Bergapten (**27**), isopimpinellin (**31**), and xanthotoxin (**32**)	Volnukhin et al. (2006)

35.6 Safety: Toxicity and Side Effects

Coumarin (**1**) itself is moderately toxic to the liver and kidneys ($LD_{50} = 275$ mg/kg) in humans but is highly hepatotoxic in rats, but less so in mice. Rodents metabolize it mostly to 3,4-coumarin epoxide, which on further differential metabolism may cause liver cancer in rats and lung tumours in mice. Humans metabolize coumarin mainly to umbelliferone (**2**), which is much less toxic than coumarin (**1**). According to the German Federal Institute for Risk Assessment the tolerable daily intake (TDI) of coumarin (**1**) is 0.1 mg/kg body weight. However, it was suggested that a higher intake for a short period might not be dangerous. However, it has been shown that even a small quantity of coumarin may cause reversible liver damage in humans. Therefore, isolated coumarins must not be added to foods. To flavor foods, coumarin-containing plant parts may be used. For cinnamon-containing foods, new maximum permissible coumarin levels have been in place in the European Union since 2011. There are no limit values for cinnamon (*Cassia cinnamon*) as a spice. Cinnamon-based food supplements may contain high quantities of *Cassia cinnamon*, and the consumers should always limit their consumption to moderate levels. In the case of frequent use of large amounts of cinnamon as a spice in cooking, one should use Ceylon cinnamon that has low levels of coumarins.

Phototoxicity of several furanocoumarins, e.g., psoralen (**13**), bergapten (**27**), and xanthotoxin (**32**), found in the food plants, mainly from the families Apiaceae and Rutaceae, is well documented (Wagstaff 1991). Photoallergic contact dermatitis to xanthotoxin (**32**), isolated from fig, was demonstrated in a human trial using positive

patch test reactions observed in 12 of 47 patients. However, interestingly, **27** was negative in that trial. Histopathological findings on biopsies from positive photopatch tests to **32** confirmed dermatitis (Bonamonte et al. 2010).

Efficacy, safety, and toxicity of coumarins present in diets, like any other compounds, depend on their type and the amounts consumed. Yordi et al. (2017) utilized the in silico technology to study the genotoxicity of the major dietary coumarins and to identify the structure-activity relationships and genotoxic structural alerts. They carried out a virtual screening using a clastogenic model and different software, e.g., MODESLAB, ChemDraw, and STATISTIC. Simple coumarins and furanocoumarins, e.g., xanthotoxin (**32**), isopimpinellin (**31**), esculin (**18**), scopoletin (**9**), scopolin (**23**), and bergapten (**27**), were classified as active in this screening. In silico genotoxicity was mainly predicted for coumarins found in beer, sherry, dried parsley, fresh parsley, and raw celery stalks.

Furanocoumarins, mainly psoralen derivatives, are the major components of grapefruit juice, and these compounds are mainly responsible for the interactions with conventional drugs (Fuhr 1998). Simultaneous intake with grapefruit juice is known to increase the concentrations of many drugs in humans, e.g., felodipine, nitrendipine, nisoldipine and saquinavir. The effect is thought to be mediated by suppression of the cytochrome P450 enzyme CYP3A4 in the small intestinal wall, which leads to diminished first-pass metabolism with higher bioavailability and increased maximal plasma concentrations of substrates of this enzyme. Therefore, it is advisable that patients should not drink grapefruit juice when taking a drug that is extensively metabolized the cytochrome P450 enzyme CYP3A4.

As a part of the investigation focusing on the inhibitory effects of angelicin (**12**) and psoralen (**13**), Lu et al. (2014) also studied their toxicity and side effects. After administration of high doses of these coumarins, toxic reactions such as writhing, lassitude, and hypoactivity were observed. Kidney histopathology demonstrated tubulointerstitial dilatation and congestion and inflammatory cell aggregation in the renal intercellular space. However, none of these compounds caused any significant toxic side effects to the bone marrow or other organs such as the heart, the lung, the liver, and the spleen. Hepatotoxicity of both **12** and **13** in rat and mice models was reported by Wang et al. (2018b); these compounds could induce the toxic reactions of liver and other organs in rats, while mice were not sensitive to these compounds. It was suggested that these compounds could cause liver injury, possibly through inhibiting bile acid excretion in the liver, leading to the accumulation of toxin in hepatocytes. It was also shown that **13** could induce developmental toxicity in zebrafish embryos/larvae mediated by oxidative stress, apoptosis, and energy metabolism disorder (Xia et al. 2018).

Singh et al. (2018) found no toxicity symptoms in osthol (**10**)-treated mice while studying its analgesic and anti-inflammatory properties. However, in a study conducted by Shokoohinia et al. (2017), it was shown that this coumarin was a moderately toxic substance when administered intraperitoneally (i.p.), and renal function impairment was caused by this compound in a rodent model. In that study, for acute toxicity, single doses

of osthol (100, 500, and 1000 mg/kg) were administered i.p. to mice, and the mice were then monitored for 14 days, whereas for subchronic toxicity, it was administered orally to rats at doses of 5, 25, and 50 mg/kg/day for 45 days.

The safety of oral administration of auraptene (**11**) was evaluated in rats in acute and subacute toxicity by evaluating clinical signs, hematology, biochemical factors, pathological changes, and immunotoxicity (Vakili et al. 2017). Acute administration of **11** in doses of 125–2000 mg/kg body weight had no mortality or clinical signs in a period of 2 days. To assess subacute toxicity, coumarin **11** was administrated for 28 days by oral gavage in doses of 125 and 250 mg/kg. Considerable differences in the hematological and biochemical data of the treated and untreated groups were observed, albeit almost all hematological differences were within normal reference ranges. While subacute administration of **11** exhibited no toxic histopathological effects on organ tissue, the assessment of immunotoxicity revealed no significant differences between treatment and untreated groups.

Kleiner et al. (2002) studied the probable systemic toxicity of imperatorin (**34**) and isopimpinellin (**31**) in a mouse model following oral administration and did not find any significant changes in blood clotting, renal or liver functions, and histopathology of the lungs and the kidneys. Nonetheless, a considerable increase in liver weight accompanied by cytoplasmic vacuolation of hepatocytes.

35.7 Marketed Products

The major marketed coumarin-containing food plant product is cinnamon, which is marketed as ground cinnamon, cinnamon sticks, spice mixes (such as pumpkin pie spice, curry mixes, Chinese five spice), breakfast cereals, baking mixes (muffin and cake mixes), baked goods (cookies, granola/breakfast bars), baby foods (such as infant cereals and purées) and dried tea (Ballin and Sorensen 2014).

There are also several medicinal products where the main active ingredient is dietary coumarin(s), available in the market. For example, psoralen plus UVA (PUVA) is an effective therapy for mycosis fungoides, and a modified formulation, "Turban PUVAsol," is for the treatment of alopecia areata. Similarly, Wenshen Zhuanggu Formula (WSZG), which contains a cocktail of psoralen (**13**), isopsoralen (**12**), bergapten (**27**), xanthotoxin (**32**), osthol (**10**), and imperatorin (**34**), is a traditional Chinese medicine (TCM) prescription used in clinics for adjuvant treatment of breast cancer bone metastases in China (Chen et al. 2018c). A similar therapy, called PUVA bath therapy, which incorporates a mixture of three coumarins, bergapten (**27**), isopimpinellin (**31**), and xanthotoxin (**32**), is available in the market for the treatment of psoriasis (Volnukhin et al. 2006), and Yuanhu Zhitong tablet, where imperatorin (**34**) and isoimperatorin (**38**) are the main active ingredients, is prescribed for various types of pain management. There are several xanthotoxin (32)-containing prescription products/formulations available in the market, and some of those are presented below (Table 5).

Table 5 Examples of some commercially available xanthotoxin (**32**)-containing prescription products (source: DrugBank at https://www.drugbank.ca/drugs/DB00553)

Products	Dosage forms	Quantity (Each unit)	Routes of administration	Providers
Methoxsalen	Liquid-filled capsule	10 mg	Oral	Actavis Pharma Inc.
8-Mop	Gelatin coated capsule	10 mg	Oral	Valeant Pharmaceuticals North America
Oxsoralen	Lotion	10 mg/mL	Topical	Valeant Pharmaceuticals North America
Oxsoralen-Ultra	Liquid filled capsule	10 mg	Oral	Valeant Pharmaceuticals North America
Uvadex	Injectable solution	10 μg/mL	Extracorporeal injection	Therakos
Ultra Mop Lotion 1%	Liquid	1%	Topical	Canderm GP

35.8 Patents

Patent publications (2015–2016) describing coumarins and their derivatives have been reviewed by Detsi et al. (2017), which included synthesis, biological evaluations *in vitro/in vivo*, e.g., antiviral, anticancer, cytotoxic, antioxidant, and anti-inflammatory protocols, and a discussion on several pharmaceutical applications and pharmaceutical compositions of coumarins. However, most of the coumarins covered in that review are synthetic analogues, most often inspired by natural coumarins, e.g., angelicin (**12**), bergapten (**27**), psoralen (**13**), scopoletin (**9**), scoparone (**22**), and umbelliferone (**2**), which are major components of various dietary plants (Detsi et al. 2017). For example, Bianchi et al. (2009) patented (US patent no. US 07572827) the use of angelicin (**12**) and of its structural analogues for the treatment of thalassemia. Similarly, pharmaceutical product (patent number: US 08518460) containing osthol (**10**), and several furanocoumarins, e.g., bergapten (**27**), imperatorin (**34**), isopimpinellin (**31**), xanthotoxin (**32**), and xanthotoxol (**33**), to treat psoriasis was patented in 2013 (Yang 2013). However, the major active ingredient in this product is osthol (**10**) accounting for about 90% by weight of the active ingredients.

35.9 Perspectives

Coumarins have been reported from a variety of edible plants, fruits, and vegetables, and reports related to economically and commercially important dietary plant materials that contain high amounts of various coumarins are also available. While a vast majority of bioactivity data on dietary coumarins are based on *in vitro* studies, and a good number of publications are available reporting *in vivo* studies involving animal models, human trial reports are rather limited. Thus, further well-designed human studies involving coumarins present in dietary plants are needed. In recent years, in

silico pharmacology has progressed significantly with the phenomenal progress in computing and artificial intelligence (AI) (Sarker and Nahar 2018); this can help better understand various mechanisms involved in relation to several pharmacological properties of dietary coumarins discerned from *in vitro* and *in vivo* studies carried out to date and to establish their role in human health benefits.

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Lignans in Diets

36

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Contents

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Abstract

Lignans are a large group of natural products consisting of dimers of phenyl propane units. They are found in diverse forms distributed in a variety of plants. Owing to their biological activities ranging from antioxidant, antitumor, anti-estrogenic, antivirus, anti-inflammatory to antiviral properties, they have been used for a long time both in ethnic and in conventional medicine. In particular, it may prevent hormone-dependent diseases, such as breast cancer, prostate cancer, and benign prostatic hyperplasia. However, many important scientific problems have not been constrained. This chapter has systematically reviewed the bioactive constituent, classification, distribution, bioavailability, metabolism, bioactivities, human health, application, and safety aspects of lignans. And lastly, a prospective of future studies on lignans is elucidated.

Keywords

Lignan · Bioactivity · Bioavailability · Metabolism · Human health

36.1 Introduction

Lignans are a family of secondary metabolites widely distributed in plants and human food sources. The term lignan was coined by Haworth in 1936 to describe a group of phenylpropanoid dimers in which C6-C3 units are linked by the central carbon of their propyl side chains (Stasevich et al. 2009). These compounds show dimeric structures formed by a β, β′-linkage between two phenyl propane units with a different degree of oxidation in the side chain and a different substitution pattern in the aromatic moieties. Similar to the ecological functions of several other secondary metabolites, lignans represent a means of protection against herbivores and microorganisms for the plants that synthesize them. For nomenclature purposes, the C6-C3 unit is treated as propylbenzene and numbered from 1 to 6 in the ring, starting from the propyl group, and with the propyl group numbered from 7 to 9, starting from the benzene ring. With the second C6-C3 unit, the numbers are primed. When the two C6-C3 units are linked by a bond between positions 8 and 8′, the compound is named as a "lignan" (Teponno et al. 2016).

Lignans are bioactive, non-nutrient, non-caloric, phenolic plant compounds that are found in diverse species in the plant kingdom including members of pteridophytes, gymnosperms, and angiosperms (Fuss 2003). In angiosperms, lignans have been isolated from members belonging to Asterales, Scrophulariales, Lamiales, Solanales, Apiales, Sapindales, Aristolochiales, Piperales, Laurales, Malvales, Malpighiales, and Magnoliales orders in the division Magnoliophyta (Stasevich et al. 2009). The enterolignans (sometimes referred to as mammalian lignans) are metabolites of food lignans produced by human intestinal bacteria. They have been identified in human urine and plasma. It is known that lignans have remarkable ecological functions in plants, providing protection against herbivores and

microorganisms. The consumption of foods rich in lignans has potential to decrease the risk of cancers. During its long research history, this family has exhibited attractive pharmacological activities, such as antibacterial, antiviral, antitumor, antiplatelet, phosphodiesterase inhibition, 5-lipoxygenase inhibition, HIV reverse transcription inhibition, cytotoxic, antioxidant, immunosuppressive, and antiasthmatic properties (Fang and Hu 2018).

The following is an overview on the current status of research on lignans in terms of bioactive constituents, bioavailability, metabolism, bioactivities, application, toxicity, and side effects.

36.2 Bioactive Constituents of Lignans

Lignans are one of the largest groups of naturally occurring phenols in the plants. The lignans are derived from the shikimic acid biosynthetic pathway. Traditionally, lignans are divided into two classes: classical lignans and neolignans. It should be noted that the term lignan in the literature refers to classical lignans in most cases. Regarding the classification of classical lignans, four different types are reported. The first one arranged classical lignans into three subgroups: acyclic lignan derivatives, arylnaphthalene derivatives, and dibenzocyclooctadiene derivatives (Chang et al. 2005). The second type includes six subgroups: dibenzylbutanes, dibenzylbutyrolactones, arylnaphthalenes, dibenzocyclooctadienes, substituted tetrahydrofurans, and 2,6-diarylfurofurans (Pan et al. 2009). The third one includes seven subgroups of lignan scaffolds: cyclobutanes, tetrahydrofurans, furofurans, dibenzylbutanes, aryltetralins, cycloheptenes, and dibenzocyclooctadienes (Albertson and Lumb 2015). The fourth one is comprised of eight subgroups: furofuran, furan, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene, and dibenzylbutyrolactol (Fig. 1) (Satake et al. 2015).

In a recent review, Teponno et al. (2016) clearly described the plant source, isolation, structure, bioactivities, and synthesis of bioactive lignans. At present, 131 dibenzocyclooctadiene-type lignans have been isolated, especially from different species of *Schisandra* and *Kadsura,* such as marlignans A–L and M–S from *S. wilsoniana*; tiegusanins A–M from *S. propinqua*; neglignans A, B, and E–G, neglectalignans A–D, and neglschisandrins B, E, and F from *S. neglecta*; arisanschinins F–L from *S. arisanensis*; schilancifolignans A–C from *S. lancifolia*; kadsuphilols I–M from *Kadsura philippinensis*; and 14-*O*-demethyl polysperlignan D from *K. coccinea* (Teponno et al. 2016).

There are 16 types of lignans isolated from sesame. Most of them are fat-soluble aglycones and therefore elute into the oil on extraction. The remaining are glycosylated and have been isolated from the oil-free meal. The major aglycone lignans are sesamin and sesamolin (Dar and Arumugam 2013). Sesamol, sesaminol, sesamolinol, pinoresinol, matairesinol (MAT), lariciresinol, and episesamin form minor aglycones of sesame oil (Liu et al. 2006). The lignan glycosides include mono-, di-, and triglucosides of sesaminol, sesamolinol, and pinoresinol (Moazzami

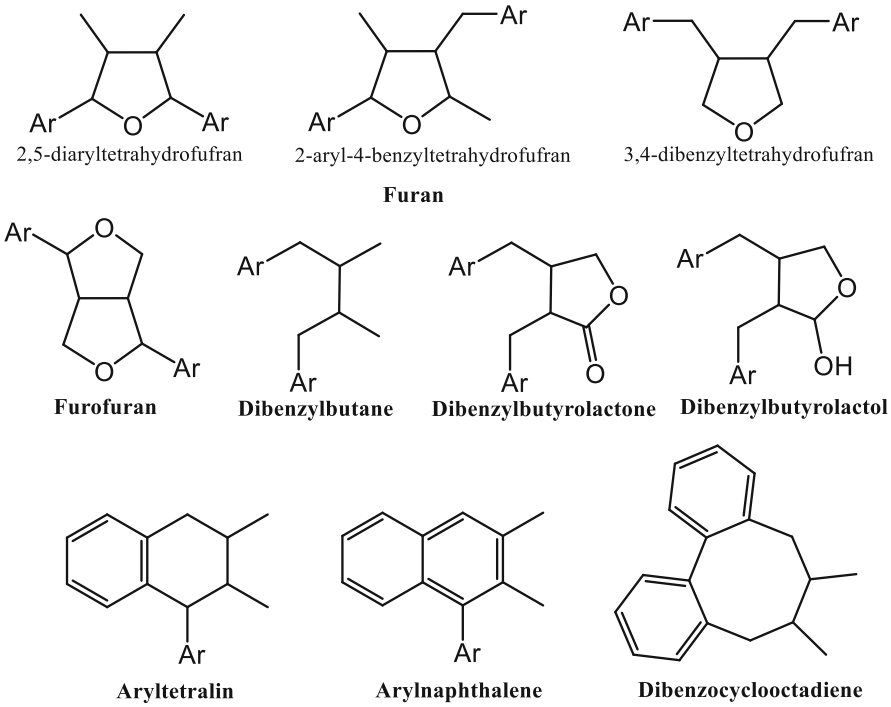

Fig. 1 Subtypes of classical lignans (Ar = aryl)

et al. 2006). Sesaminol triglucoside, sesaminol diglucoside, and sesaminol monoglucoside are the most abundant lignan glycosides in sesame. *Sesamum alatum*, a species with winged seeds, is said to be devoid of both sesamin and sesamolin but has a novel furofuran lignan, 2-episesalatin (Kamaleldin and Yousif 1992). Three additional lignans, namely, saminol, episesaminone-9-*O*-β-D-sophoroside, and semamolactol, have been detected in the perisperm of *Sesamum indicum* (Grougnet et al. 2012). Episesaminone, a furanoketone, was characterized in part via hemisynthesis from sesamolin. Recently these authors have isolated two new lignans, namely, sesamolinol-4′-*O*-β-D-glucoside and disaminyl ether (samin dimer), from sesame seeds (Grougnet et al. 2012).

36.3 Bioavailability and Metabolism of Lignan

36.3.1 Lignan Bioavailability

A prerequisite for investigating lignan bioavailability is to accurately determine their occurrence in foods and to estimate their intake in human populations. Although flaxseed is the main source of lignans (approximately 4 mg/g dried mass), a variety of cereals, fruits, vegetables, legumes, and beverages also contain

lignans in substantial concentrations (10 ng to 400 μg/g) (Milder et al. 2005). Thus, lignans are found in a wide range of foods consumed daily in Western countries. Secoisolariciresinol diglucoside (SDG), its aglycone secoisolariciresinol (SECO), and MAT are the most frequently studied dietary lignans.

A recent study reported that the absolute oral bioavailability of schisandrin B in male and female rats was about 55.0% and 19.3%, respectively. Further, micronization technology effectively improved the bioavailability of schisandrin B (two-fold) when compared with the untreated preparation (Wang et al. 2017). Sa et al. (2015) reported that the absolute bioavailability of schisantherin A in nanoemulsion formulation was significantly enhanced from 4.3% to 47.3%. Among the various *Schisandra* lignans, schisandrol A, schisandrin B, and schisandrin C appeared to be poorly absorbed in intestinal cells. Previous studies reported that schisandrin A appears to be one of the most relatively absorbed lignans (Wang et al. 2011). In a self-emulsifying drug delivery system using oleic acid, Polysorbate 20, and Transcutol P, the bioavailability of schisandrin and schisandrin B was 292.2% and 205.8%, respectively (Shao et al. 2010).

36.3.2 Lignan Metabolism

Absorption of plant lignans and bioconversion of plant lignans to enterolignans and their subsequent absorption vary greatly from person to person. Lignans are present in plants both as aglycones (without sugars) and as glycosides (with sugars) (Julia et al. 2010). At present, only in flaxseed has SECO been found as a lignan oligomer. Lignan glycosides are absorbed in the gastrointestinal tract after metabolism by intestinal bacteria to lignan aglycones and the enterolignans (enterolactone (EL) and enterodiol (ED)), which are formed from them. The extent of hydrolysis to release the lignans from the sugars (and in flax from the oligomer), the formation of enterolignans, and the bioavailability of these compounds vary quite significantly from person to person. Due to these differences in metabolism in the gastrointestinal tract, lignan intake is an imperfect measure of tissue exposure (Clavel et al. 2006).

36.3.2.1 Lignan Metabolism in the Gut

Lignans originate from cinnamic acid derivatives which are related biochemically to the metabolism of phenylalanine. Chorismic acid is transformed into prephenic acid via a Claisen rearrangement, which transfers the phosphoenolpyruvate-derived side chain so that it becomes directly bonded to the carbocycle, and thus builds up the basic carbon skeleton of phenylalanine. Decarboxylative aromatization of prephenic acid yields phenylpyruvic acid, and pyridoxal phosphate-dependent transamination leads to L-phenylalanine (Dar and Arumugam 2013). The biosynthesis of coniferyl alcohols is initiated with deamination of phenylalanine by phenylalanine ammonia lyase to form cinnamic acid, which is then hydroxylated by a P450 enzyme, cinnamate-4-hydroxylase, to form *p*-coumaric acid. Coniferyl alcohol is derived from the reduction of coumaric acid via coenzyme A ester to an aldehyde, which is further reduced in the presence of a NADPH molecule. Formation of the

coenzyme A ester facilitates the first reduction step by introducing a better leaving group ($CoAS^{-}$) for the NADPH-dependent reaction (Vogt 2010).

Lignan glycosides, such as the flax SDG ester-linked complex and the sesame seed sesamolin triglucoside, are hydrolyzed by some of the anaerobic microbes in the gut to lignan aglycones (Jan et al. 2009; Kim et al. 2006). The free lignans are then converted into enterolignans through a series of metabolic reactions by various gut bacteria (Clavel et al. 2006). The efficiency of conversion depends on many factors and differs considerably from one individual to another. The metabolism of the lignans in the tissues is influenced by genetic factors, but as yet these are not well understood (Dagmar et al. 2010; Low et al. 2007).

The predominant plant lignan compound in foods, SDG, is metabolized in the gut to SECO, then to the enterolignan ED, and finally to EL, but the conversion is never 100%. The plant lignan MAT is metabolized directly in the gut to the enterolignan EL. In an in vitro fecal microflora metabolism system, lariciresinol was completely converted in 24 h into the enterolignans EL (46%) and ED (54%), whereas other plant lignans were incompletely converted, i.e., MAT (62%), SDG (72%), and pinoresinol diglucoside (55%). All four were metabolized to EL, in part, but SECO and pinoresinol diglucosides were converted to ED (50% of the SECO and 32% of the pinoresinol total doses) and then in small amounts to EL (21% of the SECO and 19% of the pinoresinol total doses) (Heinonen et al. 2001). Other lignans that are metabolized to EL include arctigenin, 7-hydroxymatairesinol, sesamin, and syringaresinol (Heinonen et al. 2001; Peñalvo et al. 2005). Smeds et al. (2006) found cyclolariciresinol, lariciresinol, and MAT but not SECO in serum samples from a Finnish population. Peñalvo et al. (2004) determined the presence of cyclolariciresinol, lariciresinol, MAT, pinoresinol, as well as anhydrosecoisolariciresinol, 7′-hydroxymatairesinol, SECO, and sesamin in plasma of Finns after the ingestion of sesame seeds (50 g).

The enterolignans ED and EL have been detected in the blood and urine of both humans and animals, but only small amounts of the plant lignans cyclolariciresinol, lariciresinol, MAT, pinoresinol, SECO, and syringaresinol have been found in human urine (Tarja et al. 2003). In contrast, lignins are thought to be largely inert and not absorbed in the human gut due to their polymeric nature. It is possible that they are dietary precursors of enterolignans, but the ability of gut bacteria to transform and metabolize lignins into enterolignans has yet to be demonstrated in human studies (Begum et al. 2004). This possibility is worth pursuing since conversion of food lignins to lignans might explain the relatively high concentrations of enterolignans in biofluids compared to lignan intakes (Horner et al. 2002).

EL is the main circulating enterolignan; therefore, serum EL levels and urinary EL excretion are used as biomarkers for plant lignan intakes. However, these are imperfect surrogates. Differences between lignan intakes and EL production may arise because of variations in the composition of the gut microflora, conversion of some lignans into other compounds, intestinal transit time, the metabolic half-life of EL, the redox state of the colon, the types of lignans present in the diet, and the use of antibiotics (Kilkkinen et al. 2001; Clavel et al. 2006).

36.3.2.2 Systemic Metabolism

Once they are formed from the parent plant lignans by gut microbiota, the enterolignans ED and EL are absorbed through the colonic barrier (Jansen et al. 2005), and most are conjugated to glucuronides in the tissues. They are usually detectable in the blood 8–10 h after dietary intake (Clavel et al. 2006). In a recent study, some plant lignans (anhydrosecoisolariciresinol, 7′-hydroxymatairesinol, cyclolariciresinol, lariciresinol, MAT, pinoresinol, SECO, and sesamin) were rapidly absorbed in the small intestine and appeared in the systemic circulation within an hour after the ingestion of sesame seeds (Peñalvo et al. 2005). The mechanisms responsible for the uptake of plant lignans in the small intestine are still unknown (Lampe et al. 2006). The pharmacokinetic characterization of lignans is an under-researched area that must be pursued if further insights are to be gained about the actual lignan compounds providing putative health benefits.

The enterolignans either enter enterohepatic circulation or are excreted in the urine, usually as glucuronides and sulfate esters (Knust et al. 2006). Some free lignans and aliphatic or aromatic hydroxylated metabolites from hepatic metabolism may also be excreted (Knust et al. 2006; Lampe et al. 2006). One study found that the total amount of EL and ED detected in the urine was up to 40% of the ingested dose (0.9 mg/kg body wt, average 60–66 mg) of SDG and the majority of it was excreted within 2 days (Kuijsten et al. 2005).

The enterohepatic recirculation of SECO, sesame lignans, and enterolignans is significant. In general, lignans permeating the gastrointestinal mucosa are likely to undergo extensive first-pass metabolism by phase II enzymes, resulting in glucuronidation or sulfation, either in the mucosa or in the liver prior to their appearance in the systemic circulation. Glucuronides and sulfates of SECO, EL, and ED may undergo enterohepatic recirculation or simply be eliminated in the bile or urine (Jan et al. 2010; Liu et al. 2006).

Lignan intakes, as evaluated with available food composition data and dietary records or even with biomarkers, are such imperfect estimates of exposure that they may obscure diet-disease relationships. In the lignan food frequency questionnaire validation study, conducted by Horn-Ross et al. (2006) using only MAT and SECO, the correlations with urinary total ED and EL were only 0.16. In the food frequency questionnaire validation study of Bhakta et al., the correlation of MAT and SECO "true intake" with plasma EL was only 0.11. Since several other lignans are present in the diet and can be converted to EL or ED at varying rates, and some lignans are absorbed without conversion, such low correlations are not surprising. However, these problems do point to the need to improve dietary assessment methodology for these compounds.

36.4 Lignan Bioactivities

Lignans individually as well as in combination have been found to exhibit varied biological activities. What mechanisms underlie the activity of either the naturally occurring lignans or their derived metabolites? The antioxidant activity and the

estrogenic and antiestrogenic functions of lignans, as well as the influence of lignans on hormone metabolism and availability and on gene expression and/or enzyme activity, could all explain the effects of lignans. Some examples are provided in this section. Mainly ED and EL are responsible for these functions; thus the transformation of plant lignans by intestinal microbiota might be essential for these functions to be manifested (Landete 2012).

36.4.1 Antioxidant Activity

Some authors described the structure-activity relationships of lignans from *S. chinensis* in connection with antioxidant activity. Lee et al. (1999) studied the structure-activity relationships of lignans and their derivatives from *S. chinensis* as platelet-activating factor antagonists. In their study, 6,7- dehydroschisandrol A, a dibenzocyclooctadiene lignan, showed the strongest activity. The higher activity of lignans was observed in the absence of an ester group at C-6, a hydroxyl group at C-7, or a methylenedioxy moiety and the presence of an R-biphenyl configuration. Yim and Ko (1999) examined the protective effects of schisandrin A, schisandrin B, and schisandrin C against myocardial ischemia-reperfusion injury. The authors found that methylenedioxy group and the cyclooctadiene ring of the schisandrin molecule play as important structural determinants in the protection against myocardial ischemia-reperfusion injury. Choi et al. (2006) investigated the structure-activity relationships of the dibenzocyclooctadiene lignans in relation to their antioxidant activity. The study showed that the exocyclic methylene functionality was important for antioxidant activity of lignans. In addition, the presence of benzoyloxy group possibly improves such effects.

In some cases, antioxidant activity appears to be responsible for the bioactivity of plant and mammalian lignans. For example, beneficial effects of SDG in cancer and lupus nephritis revealed that these beneficial effects could be due to the ability of SDG to scavenge hydroxyl radicals (Prasad 1997), showing SDG to exert powerful antioxidant activity.

The antioxidant activity of flaxseed lignans and derived metabolites is because they exert protective effects against AAPH (2, 2′, azobis (2-amidinopropane) dihydrochloride), a compound used extensively as a free radical generator (Hosseinian et al. 2007; Hu et al. 2007). The flaxseed lignan SDG and mammalian lignans ED and EL act as antioxidants against DNA damage and lipid peroxidation. Plant lignan antioxidant activity has been attributed to the 3-methoxy-4-hydroxyl substituents of SDG and SECO, versus the meta mono-phenol structures of ED and EL. Benzylic hydrogen abstraction and potential resonance stabilization of phenoxyl radicals in an aqueous environment are likely to contribute to the antioxidant activity of the mammalian lignans. These probably represent extra- and intracellular antioxidant activities of flax-derived lignans at concentrations that are potentially achievable in vivo (Hu et al. 2007).

36.4.2 Estrogenic and Antiestrogenic Functions

Lignans and their derived metabolites ED and EL act either as estrogen agonists or antagonists. The chemical structures of these bi-phenolic compounds closely resemble that of endogenous 17β-estradiol, and they exert biphasic agonistic (estrogenic) and antagonistic (antiestrogenic) activities in vitro (Sathyamoorthy et al. 1994) and in vivo (Pauliina et al. 2011; Tou et al. 1999).

Research shows that physiologically relevant EL concentrations lead to in vitro and in vivo activation of estrogen receptor (ER)-mediated events and this has generated interest because of their potential use in hormone replacement therapy and cancer prevention (Hébert-Croteau 1998).

Because of their structural similarity to 17β-estradiol, enterolignans are natural ligands of ERs and are believed to be naturally existing selective estrogen receptor modulators. They might therefore act as anticarcinogens, either through antiestrogenic actions (e.g., by competing with estradiol to bind ERs) or by initiating their own anticarcinogenic effects (e.g., by recruiting specific transcriptional coregulators to phytoestrogen-activated ERs).

The mechanism of action of enterolignans on ER has been studied, and there is evidence from human observational studies that phytoestrogens may modulate hormone levels and ER expression (Touillaud et al. 2005). It is assumed that the biological action of phytoestrogen is mediated by ERα and ERβ. The ER is a ligand-dependent transcription factor belonging to the nuclear receptor superfamily. The ER binds estrogen response elements, a 13-bp inverted repeat, through its conserved DNA-binding domain. The ER contains two transcriptional activation domains: the autonomous transcriptional activation domain, AF-1, located at the N-terminus, and the ligand-dependent activation domain, AF-2, located at the C-terminus (Green and Chambon 1988); the primary sequence of AF-2 differs significantly between ERα and ERβ. This causes different agonist/antagonist characteristics for various chemicals containing phytoestrogens, depending on their affinity for the receptors (Barkhem et al. 1998). Carreau et al. (2008) examined and compared the ability of ED, EL, and 17β-estradiol to induce the transactivation of ERα and ERβ, to modulate ERα target genes. This study indicates that enterolignans have distinct properties for the transactivation of ERα and ERβ. ED, like 17β-estradiol, induces ERα transcriptional activation through transactivation functions AF-1 and AF-2, while EL is less efficient in inducing AF-1, acting mainly through AF-2. Furthermore, ED and EL modulate ERα mRNA and protein contents as well as MCF-7 cell proliferation.

EL, at physiological concentrations, activates ER-mediated transcription in vitro with preference for ERα. The effects of EL are mediated by the ER ligand-binding domain and are susceptible to antiestrogen treatment. Penttinen et al. (2007) demonstrated that EL exerts estrogenic activity in vivo. In transgenic estrogen-sensitive reporter mice, EL induces tissue-specific estrogen-responsive reporter gene expression as well as promotes uterine stromal edema and expression of estrogen-responsive endogenous genes (Cyclin D1 and Ki67). Taken together, these data show that

EL is a selective ER agonist inducing ER-mediated transcription both in vitro in different cell lines and in vivo in the mouse uterus.

Therefore, the transformation of plant lignans by intestinal microbiota might be essential for the estrogenic and antiestrogenic activity to manifest. Lignans and their derived metabolites have also been associated with a reduction in the risk of breast cancer through estrogenic and antiestrogenic effects. As above mentioned, the estrogen agonistic effect of ED and EL may be useful for conventional hormone replacement therapy in postmenopausal women. However, Pianjing et al. (2011) suggested that due to a potential tumor growth stimulation of lignans and their ability to induce certain estrogen-related genes, oral supplementation of enterolignans should be prescribed with caution, particularly in postmenopausal women and hormone-dependent breast cancer patients.

36.5 Health Benefits

Lignans are polyphenolic compounds with a wide spectrum of biological functions including antioxidant, anti-inflammatory, and anticarcinogenic activities; therefore, there is an increasing interest in promoting the inclusion of lignan-rich foods in humans' diets. The lignans consumed by human are in fact digested by the microflora present in the intestine. The weak and antiestrogenic effects of lignans are caused by distinct transactivation activities of estrogen receptors between the enterolignans ED and EL (Brito and Zang 2018). Previous researches indicate that certain conditions including breast, colon, and prostate cancer can be reduced by dietary lignan intake and/or increased levels of EL and/or ED. Clearly, more research is needed to determine causality and to evaluate the potential role of lignans and their metabolites in metabolic profiles.

36.5.1 Colon Cancer

ERs, especially the β-type receptors, are abundant in colon cells (Konstantinopoulos et al. 2003), where they play a role in normal colon functioning (Wada-Hiraike et al. 2006); however, cancer progression is associated with a loss of ER β (Castiglione et al. 2008; Jassam et al. 2005). This, together with data from epidemiological studies showing that hormone replacement therapy protects against colon cancer (Nelson et al. 1997), would indicate that EL has the potential to protect against colon cancer. EL may exert these potential effects both during absorption from the colon lumen and when passing the intestinal cells during systemic circulation.

Treatment of human colon cancer SW480 cells with EL and ED, either alone or in combination, resulted in dose- and time-dependent decreases in cell numbers (Qu et al. 2005). Cell growth inhibition by lignan metabolites seems to be mediated by cytostatic and apoptotic mechanisms (Ayella et al. 2010).

In a case-cohort study of Danish middle-aged men and women, which gathered detailed information on diet and lifestyle factors, Kuijsten et al. (2006) investigated

the association between plasma enterolignans and the incidence of colon and rectal cancer. The authors observed a substantial reduction in colorectal adenoma risk among subjects with high plasma concentrations of enterolignans, in particular, ED. Recently, Johnsen et al. (2010) examined the association between plasma EL concentration and incidence of colon and rectal cancer in 57,053 participants aged 50–64. They concluded that higher EL levels are associated with lower risk of colon cancer among women and higher risk of rectal cancer among men.

36.5.2 Breast Cancer

Some studies show that the administration of plant lignans, which are further metabolized to EL or EL as such, inhibit or delay the growth of experimental mammary cancer (Saarinen et al. 2010). The mechanisms underlying the anti-carcinogenic action of EL are not yet fully understood, but there is intriguing evidence for EL as a modulator of estrogen signalling. Sesamin is converted to the phytoestrogens and ELs. The phytoestrogens are known to play protective role against breast cancer (Peñalvo et al. 2005). Therefore, lignans and their derived metabolites have been also associated with a reduction in the risk of breast cancer through an estrogenic and antiestrogenic effect.

The results of the large prospective study of French women done by Touillaud et al. (2007) showed that higher dietary intakes of lignans were associated with a reduction in the risk of postmenopausal breast cancers. However, epidemiological studies that examined whether lignans protect against breast cancer have yielded inconsistent results. In this respect, Buck et al. (2010) conducted meta-analyses on the association between lignans and breast cancer risk. The meta-analyses included 21 studies (11 prospective cohort studies and 10 case-control studies), in which high lignan intake was associated with a significant reduction in breast cancer risk in postmenopausal women. Breast cancer risk was also inversely associated with enterolignan exposure but not with blood or urine EL concentrations.

36.5.3 Prostate Cancer

Lignans and their derived metabolites are believed to be partly responsible for growth inhibition of human prostate cancer cell lines (Demark-Wahnefried 2001). Morton et al. (2015) associated higher EL levels in prostatic fluid with a low risk of prostate cancer. In a small clinical study, prostate cancer cell proliferation decreased and apoptosis increased in men fed 30 g of flaxseed per day (Demark-Wahnefried et al. 2001). Other work by these authors further supports the role of flaxseed in combination with a low-fat diet as a means to control prostate growth (Demark-Wahnefried et al. 2004). In the study, prostate-specific antigen levels and cell proliferation both decreased from baseline after only 6 month on the dietary regime.

EL has been shown to inhibit prostate cancer growth and development, but the mechanistic basis for its anticancer activity remains largely unknown. Activation

of insulin-like growth factor-1 receptor signalling is critical for prostate cancer cell growth and progression (Chen et al. 2009).

36.5.4 Intestinal Cancer

Results by Pajari et al. (2006) demonstrate that MAT or SECO do not prevent intestinal carcinogenesis in MIN mice and that MAT may have adverse effects on intestinal carcinogenesis. The number of intestinal adenomas in the MIN mouse model is not related to plasma EL levels nor is it associated with the levels of intestinal lignans (Oikarinen et al. 2005).

36.5.5 Menopausal Symptoms

Phytoestrogens may be of use in ameliorating some menopausal symptoms. Results by Wu et al. (2006) suggest that sesame ingestion benefits postmenopausal women by improving blood lipids, antioxidant status, and possibly sex hormone status. On the other hand, epidemiological and pharmacological studies have shown that ED, and particularly its oxidation product EL, have preventive effects on osteoporosis and menopausal syndrome (Lemay et al. 2002).

36.5.6 Cardiovascular Disease

Flaxseed can protect against atherosclerotic plaque deposition in carotid arteries and shows anti-atherosclerotic effects in the aorta. The aforementioned authors show that dietary flaxseed can improve endothelium-dependent vascular relaxation in the presence of a high-cholesterol diet. Lignan intake may have an important protective effect against human vascular disease. Later, Dupasquier et al. (2007) demonstrated how dietary flaxseed can inhibit the atherogenic effects of a high-cholesterol diet in the LDLrKO mouse. However, the results by Kuijsten et al. (2009) do not support the hypothesis that high plasma ED or EL concentrations are associated with a reduced risk of nonfatal myocardial infarction. On the other hand, sesamin inhibits intestinal absorption of cholesterol and reduces the activity of acylCoA:cholesterol acyltransferase and 3-hydroxy-3-methylglutaryl CoA reductase in rats (Wu et al. 2006). Sesamin, at a rather low dose (65 mg/day or approximately equivalent to consuming 13 g of sesame seeds), lowers plasma cholesterol in subjects with hypercholesterolemia (Hirata et al. 1996).

36.5.7 Hepatoprotective Effects

Platelet-activating factor (PAF) has been linked to aggregation and degranulation of platelets and is an important mediator in inflammation and asthma. Plant lignans have been reported to exert anti-PAF activity (Tibiriçá 2010). Flaxseed

potentially inhibits several mechanisms associated with renal disease in lupus nephritis. Incorporating flaxseed into the diet of either an experimental mouse model of lupus, MRL/PR, or human lupus nephritis subjects demonstrated significant changes in renal and neutrophil function and plasma lipids (Westcott and Muir 2003). Doses above 30 g/day were not well tolerated mainly due to increased laxation. Use of purified SDG in the mouse model (MRL/lpr) showed that lignan was well tolerated and provided reno-protection similar to whole flaxseed (Clark et al. 2001). Ogborn et al. (2002) administered purified SDG and found that cystic change, epithelial proliferation, interstitial fibrosis, macrophage infiltration, and oxidant injury were all reduced (Ogborn et al. 2002). Recently, Moneim et al. (2014) have demonstrated how flaxseed oil may play a protective role against kidney injury.

Hepatoprotective effects have also been associated to lignans in a flaxseed supplemented diet. Hemmings and Barker (2010) found a hepatobeneficial effect of increased levels of γ-glutamyltranspeptidase (γGT) in the livers of both male and female rats. In addition, they reported that a diet with 10% flaxseed lacks long-term effects on growth, development, and behavior, is nontoxic, and may be hepatoprotective. SDG from flaxseed has been shown effective in preventing/delaying the development of type 1 and type 2 diabetes (Prasad 2002).

36.5.8 Neuroprotection

In regard to neuroprotective actions, Qin et al. (2014) reported that schisantherin A, schisandrin C, and schisandrol B were found to possess remarkable neuroprotective effects against serum and glucose deprivation injury in SH-SY5Y cells than schisandrin A, schisandrin B, and schisanhenol. The authors suggested that the number and position of a hydroxyl group and a methylenedioxy may be responsible for the neuroprotective effects of these lignans. Out of five dibenzocyclooctadiene lignans (deoxyschisandrin, gomisin N, gomisin A, schisandrin, and wuweizisu C) isolated from the methanolic extract of *S. chinensis*, deoxyschisandrin, gomisin N, and wuweizisu C markedly protected the glutamate-induced neurotoxicity in rat cortical cells (Kim et al. 2004). Song et al. (2015) studied the protective effects of schisandrin, schisantherin A, schisandrin B, and schisandrin C on amyloid-β_{25-35}- and homocysteine-induced neurotoxicity in PC12 cells. Among the four lignans, schisandrin B and schisandrin C effectively protected amyloid-β-induced neurotoxicity in PC12 cells by inhibiting the production of reactive oxygen species (ROS) and modulating the apoptotic signal pathway via Bax and caspase-3. Further, gomisins A, G, J, and N (schisandrin B) are strong inhibitors of toll-like receptor 2/4 (TLR 2/4) agonist-induced hyperneuroinflammatory responses (Young et al. 2014).

36.5.9 Anti-inflammatory Activity

Sesamin exhibited anti-inflammatory activity by inhibiting delta-5 desaturase, a key enzyme in arachidonic acid biosynthesis that leads to a reduction in the formation of

pro-inflammatory mediators (Chavali et al. 1998). An unusual tetrahydrofuran lignan from the roots of *Zanthoxylum planispinum* has also the potential anti-inflammatory effects (Su et al. 2016).

36.6 Application of Lignans in Food

36.6.1 Food Sources of Lignans

The lignan content of foods is generally low and usually does not exceed 2 mg/100 g. The exceptions are flaxseed (335 mg/100 g) and sesame seeds (373 mg/100 g), which have a lignan content a hundred times higher than other dietary sources (Julia et al. 2010). They are present in many plant families, although the types and amounts vary from one family to another. Lignans are found in whole grains (especially in the bran layer) and seeds (in the seed coat). Barley, buckwheat, flax, millet, oats, rye, sesame seeds, and wheat contain fairly high levels of lignans. Nuts and legumes are also reasonably good sources. Although in lesser amounts than in grains, lignans are present in fruits and vegetables such as asparagus, grapes, kiwi fruit, lemons, oranges, pineapples, wine, and even in coffee and tea (Kuhnle et al. 2009; Smeds et al. 2007).

In contrast to plants, there are virtually no lignans in animal foods. Minute amounts of the enterolignans ED and EL are sometimes found in animal foods (milk products) as a result of their production by bacterial metabolism in the animals' guts, but these are exceptions. Little has been done to investigate the effects of storage and processing on lignans in most foods (Kuhnle et al. 2009; Brenes et al. 2002), although it is known that the lignan content is apparently not changed considerably during the processing of flaxseed and sesame seed (Strandås et al. 2008; Wu 2007). Furofuran-type lignans are widely distributed in edible plants (flaxseed, sesame, seeds, cereal products, and *Brassica* vegetables). Sesame lignans in particular are obtained from *Sesamum indicum*, a highly prized oilseed crop cultivated widely in many countries in the east. The plant is the main source of clinically important antioxidant lignans such as sesamin, sesamolin, sesaminol, and sesamol.

36.6.2 Effect of Cooking on Lignans in Food

Plant foods or oils are used for preparing various foods like bakery products, chips, or blanched and cooked vegetables. During their production, the raw materials are heated to a lesser or greater extent, and the stability of lignans occurring in different conjugation patterns is influenced.

Roasting of sesame seeds at 200 °C for 60 min cleaved and liberated phenolic compounds (Jeong et al. 2010), and sesamolin could be degraded into sesamol (Lee et al. 2010). Infrared roasting of sesame seeds at 200 °C for 30 min also degraded sesamolin to sesamol (Kumar et al. 2010). In sesame oil the content of sesamol increased under heating conditions, while that of sesamolin decreased slightly, and

the sesamin content changed only little. For pinoresinol a high stability to thermal treatments below 180 °C was observed in olive oil (Brenes et al. 2002). However, a short microwave treatment already resulted in a small decrease (Cerretani et al. 2009). Kotsiou et al. (2009) reported that lignans like 1-acetoxypinoresinol remained unchanged during boiling or frying in olive oil. In pumpkin seeds, SECO was degraded by thermal heating with increasing roasting time (Murkovic et al. 2004). Hyvarinen et al. (2006) reported that the complex ester of SDG and SDG itself are stable in various bakery products. In flaxseeds, isolariciresinol, SECO, lariciresinol, and pinoresinol which were mainly present as esterified compounds were stable even if heated to 250 °C for 3.5 min. In contrast, pinoresinol aglycone in olive oil was degraded even at 180 °C (Carrasco-Pancorbo et al. 2010). This finding demonstrates that the type of conjugation and the matrix influence the thermal stability of lignans. To conserve a relatively high content of lignans during production of commercial foods, the raw product, the water content, and the applied temperatures have to be considered and optimized.

Moderate heating at 100 °C did not degrade the lignan aglycones and glycosides in dry foods. In contrast, heating was responsible for the better extractability of the lignans. If samples with high moisture content were heated, the degradation of the lignans in sesame seeds and rye was observed already at 100 °C. Higher roasting temperatures caused degradation of aglycones and glycosides.

36.7 Safety: Toxicity and Side Effects

A single-blind, placebo-controlled, parallel-group, and multiple oral dose study was conducted in 48 healthy subjects to investigate the pharmacokinetics and safety of multiple oral doses of sesame lignans (sesamin and episesamin). The results showed that sesamin was absorbed with a peak plasma concentration at 5.0 h. The plasma concentration of the main metabolite, SC-1, reached a peak at 5.0 h and decreased rapidly with a terminal half-life of 2.4 h. Episesamin was also absorbed with a peak plasma concentration at 5.0 h and decreased with a terminal half-life of 7.1 h. The plasma concentration of the main metabolite, EC-1, reached a peak at 5.0 h and decreased rapidly with a terminal half-life of 3.4 h. The plasma concentrations of sesamin and episesamin reached a steady state by day 7. Sesame lignans were confirmed to be safe and tolerable in healthy subjects (Namino et al. 2013). Niemeyer and Metzler (2002) reported that lignans resembled the isoflavone daidzein and differed from genistein and coumestrol, which exhibited clastogenic and gene mutagenic activity in V79 cells.

36.8 Marketed Products

Flaxseed can be incorporated into various food products to increase the intake of lignans. Traditionally, this has been done by adding flaxseed to bread either as whole seeds or in the form of ground flaxseed meal. However, the characteristic flavor of

flaxseed may limit its other applications in foods. More recently, the development of dehulling techniques has made it possible to separate a lignan-rich hull fraction from flaxseed (Hyvarinen et al. 2006), and several hull preparations are now commercially available. On the other hand, the possible presence of harmful substances such as cyanogenic glycosides and cadmium in flax has to be taken into account if the use of flaxseed in our diet is to be increased. Supplementation of bakery products with SDG isolated or enriched from flaxseed, thus, offers an attractive approach to be investigated.

36.9 Patents

Lignan Flax Seed Cakes offers consumers a delicious and simple way to consume ground flaxseed. This product combines multiple healthy ingredients, such as fruit, oats, and walnuts, with ground flaxseed to produce a well-textured and desirable food item. This comestible food product enables individuals to receive a known (controlled) quantity of flaxseed while simultaneously consuming other beneficial ingredients. The enjoyable taste makes it easy to obtain the health benefits provided by flaxseed within a consumer's diet.

The invention provides an enzyme having the lignan glycosidation activity by identifying an enzyme involved in the production of lignan glycosides, identifying an amino acid sequence of the enzyme polypeptide, and a base sequence encoding the polypeptide. Based on the information of these sequences, transformants capable of producing the lignan glycosides were prepared (Hyvarinen et al. 2006).

36.10 Perspectives

Although many of the studies reviewed suggest possible associations with dietary or biomarker measures of lignan exposure, several limitations are worth noting. More research on the food content of lignans and on food sources in relation to health outcomes in epidemiologic studies is needed. It may be that a certain threshold of intake is required and many Western populations either do not reach those levels or the appropriate foods are not assessed on research questionnaires. If possible, repeated measures of these biomarkers would benefit studies of the association between EL and chronic disease outcomes. Finally, it is of interest that most studies of lignan intake were of women, whereas all but one of the EL studies were of men. Because associations with lignans may vary by gender, more research including both men and women is needed. Future studies should employ both complete dietary intakes of lignans and serum (or plasma) enterolignan markers in high-risk groups.

The application of sesamin in auguring human health is one of the main themes of current research in medical science. Focus is required on validating the biological activities of furofuran lignans other than sesamin. Another point of concern at this juncture is to ensure the availability of sesamin in reasonable quantity for medical application, as sesame is the only major source of these lignans. Therefore, it may

be concluded that there is potential of a tremendous research on qualitative and quantitative improvement of the sesame crop for sesamin production. High-throughput analytical methods based on cell culture techniques would be a way out in advancing our knowledge on biosynthesis of sesamin both for productivity and human health.

Public acceptance of dietary products derived from transgenic organisms is limited. Nevertheless, lignans produced by transgenic hosts are chemically identical to natural ones and free from any recombinant genes or proteins. Thus, their public acceptance is expected to be more easily garnered than that of transgenic foods. Accordingly, more attention should be paid to the establishment of scaling-up and following industrialization of the lignan production systems (Satake et al. 2015). Large-scale lignan production by transgenic plants requires a closed cultivation system to prevent contamination of the environment by transgenic plants. Recently, various closed plant factories have been emerging, which completely shut off a gene flow into the outer environment and enable the transgenic plant-based molecular breeding of genes or compounds of interest under optimal and sterile conditions. Such advances in the metabolic engineering of lignan biosynthesis will surely pave the way for the conversion of conventional agricultural lignan production to innovative industrial lignan production.

36.11 Cross-References

- ▶ Antioxidants in Diets and Food
- ▶ Dietary Triterpenoids
- ▶ Lignans in Diets
- ▶ Phenylpropanoids (Phenylpropenes) in Diets

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Gingerols and Shogaols from Food

37

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Abstract

Gingerols and shogaols belong to the secondary metabolites of the representatives of Zingiberaceae family. These phenolic compounds are responsible for the pungent taste of ginger and also for a wide range of pharmacological properties of the plants. The aim of this chapter is to collate the information on their chemical structure, bioavailability, toxicity, pharmacological properties, and application as food products, nutraceutics, and drugs.

Keywords

Zingiberaceae · Ginger · *Zingiber officinale* · Gingerols · Shogaols · Pharmacological activity · Food products

37.1 Introduction

Gingerols and shogaols belong to plant-derived secondary metabolites, known to be synthesized by the representatives of the Zingiberaceae family (see Table 1), mainly by the genus *Zingiber*.

The composition of ginger varies depending on the collection area, time of collection, and condition of the rhizomes; however, generally it can be concluded that the odor of ginger rhizomes is affected by terpenes abundantly present in its tissues (e.g., β-sesquiphellandrene, α-zingiberene, β-bisabolene, (E,E)-α-farnesene, or ar-curcumene) whereas their pungent taste by the presence of gingerols and their dehydration products, namely, shogaols – all of which belong to the class of polyphenols (Koch et al. 2017; Ali et al. 2008; Semwal et al. 2015; Villaflores et al. 2010).

Metabolites of ginger and their impact on human health are of particular interest to a wide group of researchers around the world. Numerous studies have been performed on ginger extracts and on their isolates, which prove broad and comprehensive properties of this plant used extensively as a spice first in the Asian traditional medicine.

Ginger species originate from Asia, where they have been growing in mild climate zones with high humidity rate, like Indo-Malayan area or Japan. Now they

Table 1 The natural sources of gingerols and shogaols (Koch et al. 2017; Ali et al. 2008; Semwal et al. 2015; Villaflores et al. 2010)

	Compounds	Species	Family
1	Gingerols, shogaols	*Zingiber* spp. (L.)	Zingiberaceae
2	Gingerols, shogaols	*Alpinia* spp.	Zingiberaceae
3	Gingerols	*Aframomum melegueta* K. Schum.	Zingiberaceae
4	Gingerols, zingerone	*Trigonella foenum-graecum* L.	Leguminosae
5	Gingerols, shogaols	*Lycianthes marlipoensis*	Solanaceae

Table 2 Ginger-producing countries of the world (Dhanik et al. 2017; FAO Statistical Database http://www-fao.org)

	1999		2013
Years	Area (ha)	Production (tonnes)	Production (tonnes)
India	70,000	235,000	683,000
China	13,450	157,018	425,000
Nigeria	145,000	80,000	160,000
Bangladesh	7,700	39,000	69,000
Jamaica	180	620	–
Nepal	1,200	3,200	235,033
Indonesia	9,900	80,351	232,669
Thailand	–	–	140,000

are widely distributed around the world and are collected in Africa, Australia, and America (Yeh et al. 2014). These days India and China are the top producers of ginger rhizomes (see Table 2).

The application of the most widely cultivated species of ginger – *Zingiber officinale* Roscoe – in foods and medicine exceeds 2000 years. Within this time much research have been done in the field of cultivation, identification of the major constituents of its extracts, and, finally, its pharmacological activity. Ginger was proven to exhibit numerous effects on human health in traditional medicine formulations, including digestive, antimicrobial, bile production-enhancing, anti-emetic, anti-inflammatory, antirheumatic, laxative, anti-colic, rubefacient, and warming-up properties (Yeh et al. 2014; Ali et al. 2008). The aim of this chapter is to review the currently available scientific literature and to present the results of the in vivo studies on gingerols and shogaols properties on both animals and humans, the marketed products, and patents.

37.2 Bioactive Constituents

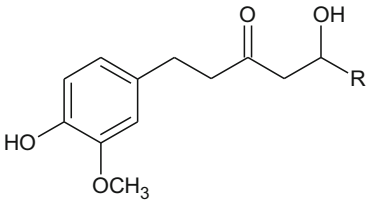
A number of pharmacological properties of ginger are responsible for its bioactive phytochemical compounds: essential oils, phenolic compounds, proteins, carbohydrates, glycosides, alkaloids, saponins, steroids, and tannins. Ingredients of ginger are numerous and differ in qualitative and quantitative content in the rhizome, depending on the place of origin and whether the raw material was dried or fresh. Terpenes are responsible for the characteristic aroma of ginger rhizomes: monoterpenes (camphene, β-phellandrene, cineole, geraniol, curcumene, citral, terpineol, borneol) and sesquiterpenes (α-zingiberene, β-sesquiphellandrene, β-bisabolene, α-farnesene, ar-curcumene). Also, an important group of secondary metabolites of ginger are polyphenolic compounds: gingerols and compounds similar to gingerols such as shogaol, paradoles, gingerdiony, and gingerdiols (Ali et al. 2008; Koch et al. 2017).

Gingerols predominate in fresh ginger rootstocks; they are homologues of 1-(3-methoxy-4-hydroxyphenyl) 3-oxo-5-hydroxyhexane, and individual compounds differ in the length of unbranched alkyl side chains; the highest number is 6-gingerol ((5S))-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one), 10-gingerol ((5S)-5-hydroxy-1- (4-hydroxy-3-methoxyphenyl) tetradecan-3-one), and 8-gingerol ((5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) dodecan-3-one), respectively. Gingerols are thermally unstable due to the presence of the β-hydroxy keto group and are easily dehydrated during drying and storage processes, forming shogaols. Among the latter compounds, which are the dehydration products of the respective gingerols, 6-shogaol ((E)-1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one), 8-shogaol ((E)-1-(4-hydroxy-3-methoxyphenyl) dodec-4-en-3-one), 10-shogaol ((E)-1-(4-hydroxy-3-methoxyphenyl) tetradec-4-ene-3-one), and 12-shogaol predominate in the dried rhizome. In turn, the paradols are structurally similar to gingerols; they are unsaturated ketones produced as a result of shogaols' biotransformation. Gingerdions, another constituent of ginger extracts, are the dehydrogenation products of hydroxylated β-ketones of gingerols and include the subgroup of 1-dehydro-gingerdiones and gingerdiols, which are ketone reduction products of gingerols (Ali et al. 2008; Dhanik et al. 2017; Tao et al. 2009). Diarylheptanoids are also present in ginger extracts. They are characterized by a backbone of the structure of 1,7-diphenylheptanes, comprising the compounds of linear or macrocyclic structures (Wei et al. 2005) (see Fig. 1).

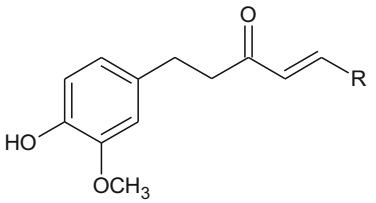
37.3 Bioavailability and Metabolism

Despite the widespread use of ginger, there is little information on the bioavailability of its compounds, especially for human use. Existing studies relate to intravenous or oral administration of the entire extract or isolated compounds on rodents and to in vitro tests in conditions similar to those existing in the human gastrointestinal tract. The poor solubility of 6-gingerol causes its weaker absorption after oral administration and rapid metabolism. There are studies suggesting the use of special solutions that deliver ginger compounds in a more efficient way (Ogino et al. 2018; Sato et al. 2017; Wang et al. 2018; Xu et al. 2016).

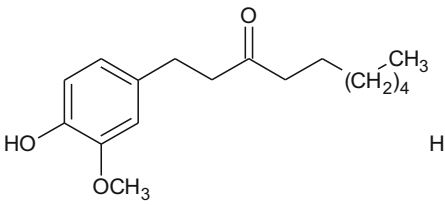
Based on studies by Jiang et al. 2008 in rats, after oral administration at a dose of 240 mg/kg of ginger extract (containing 53% (w/w) 6-gingerol), rapid absorption of 6-gingerols into the plasma, and its the maximum concentration was 4.23ug/ mL and was reached 10 min after administration. The remaining parameters were 1.77 h elimination half-life, 40.8 L/h of apparent total plasma clearance of 6-gingerol, and 18.4 L of apparent volume of distribution. Studies have shown that 6-gingerol was distributed to tissues in the brain, lungs, heart, kidneys, and liver and the highest concentration was determined in the gastrointestinal tract. Work of Arablou and Aryaeian 2018 suggests that after oral administration to rats, 6-gingerol was converted into glucuronide conjugates and was excreted in the bile and its polar metabolites in a small amount into the urine. In the same work, one study on healthy



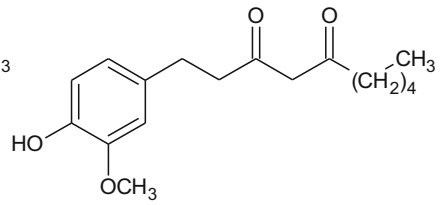
6-Gingerol [R=(CH2)4CH3]
8-Gingerol [R=(CH2)6CH3]
10-Gingerol [R=(CH2)8CH3]



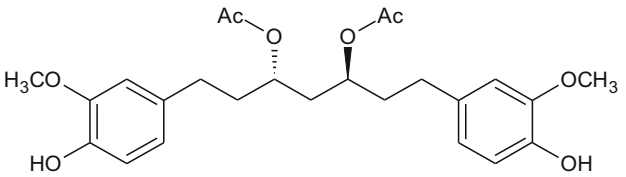
6-Shogaol [R=(CH2)4CH3]
8-Shogaol [R=(CH2)6CH3]
10-Shogaol [R=(CH2)8CH3]



6 - paradol



[6] - gingerdione



(3S,5S) - 3,5 - diacetoxy - 1,7 -bis (4-hydroxy-3-methoxy) -heptane

Fig. 1 Chemical structures of the various gingerol and shogaol analogues isolated from ginger rhizome

people, receiving various doses of 100 mg to 2 g of ginger, was described. The results of this experiment showed that the main ginger compounds, 6-, 8-, and 10-gingerol and 6-shogaol, were quickly absorbed, but they were present in the serum in the forms of glucuronide and sulfate conjugates – not their free forms.

After intravenous administration of 6-gingerol into rats at a dose of 3 mg/ kg, the compound was rapidly removed from the plasma, and its total half-life was 7.23 min, the total clearance 16.8 mL/min /kg, and the binding to serum proteins 92.4%. The elimination half-life of 6-gingerol in rats with damaged liver was significantly increased, thus indicating that 6-gingerol is mainly excreted by the liver. The major 6-gingerol metabolites have been identified as 6-gingerol diastereomers by means of GC-MS. In other studies using high-performance liquid chromatography (HPLC), the main compound gingerols administration was (S)-[6]-gingerol-40-*O*-*β*-glucuronide, and the metabolites in the urine were vanillic acid, ferulic acid (S)-(+)-

4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl)-acetanoic acid, 4-(4-hydroxy-3 methoxyphenyl) butanoic acid, 9-hydroxy-[6]-gingerol, and (S)-(+)-[6]-gingerol (Ali et al. 2008). Research by Nakazawa and Ohsawa (2002) suggests that both intestinal flora and enzymes in the liver play an important role in the metabolism of [6]-gingerol (Arablou and Aryaeian 2018; Semwal et al. 2015; Ali et al. 2008; Jiang et al. 2008; Mukkavilli et al. 2017; Xu et al. 2016; Tao et al. 2009).

6-Shogaol similar to 6-gingerol is rapidly absorbed and eliminated after oral administration in rats, and in blood it is transformed into glucuronide forms, which significantly reduces its bioactivity. Currently, 28 metabolites of 6-shogaol were identified in mouse feces and urine, including the following structures: (1E, 4E)-1-(4′-hydroxy-3′-methoxyphenyl)-deca-1,4-dienes-3-one (6-dehydroshogene), (E)-1-(4′-hydroxy-3′-methoxyphenyl)-dec-1-en-3-one (6-dehydroparadol), 1-(4′-hydroxy)-3′-methoxy-phenyl)-decan-3-one (6-paradol), 1-(4′-hydroxy-3′-methoxy)-decan-3-ol, and 1-(4′-hydroxy-3′-methoxyphenyl)-deca-4-ene-3-ol (Kou et al. 2018).

37.3.1 Biosynthesis

The basic synthesis pathway of 6-gingerol proposed and explained by Denniff et al. (1981) and Denniff et al. (1980) starts from dihydroferulic acid and hexanoic acid. The authors of this theory described the conversion of phenylalanine to dihydroferulic acid, which participates in the biological reaction of Claisen with malonate and hexanoate to form 6-dehydrogingerdione, further transformed into 6-gingerol.

Ramirez-Ahumada et al. (2006) proposed an alternative route of 6-gingerol synthesis in which the following enzymes have a significant contribution: ammonia lyase, p-coumaroyl shikimate transferase, p-coumaroyl quinate transferase, caffeic acid O-methyltransferase, and caffeoyl-CoA-O-methyltransferase (Fig. 2).

37.4 Bioactivities (Animal Aspects)

Ginger in Hindu mythology has been described as a plant helping people, plants, and animals. It belongs to one of the oldest spices and medicinal herbs. The latest and more frequent studies confirm the possibility of using ginger in many diseases. Here below the activity of polar extracts of ginger and of its single phenolic compounds are presented as drug candidates targeting a wide range of therapeutical application.

37.4.1 Antiemetic Properties

The mechanism of antiemetic action of ginger extracts remains uncertain and not fully understood. There are many theories explaining this direction of *Z. officinale* activity. One of them performed in vivo on animal models (guinea pig) suggests that the ginger extract and its single constituents (6-gingerol, 6-shogaol, and

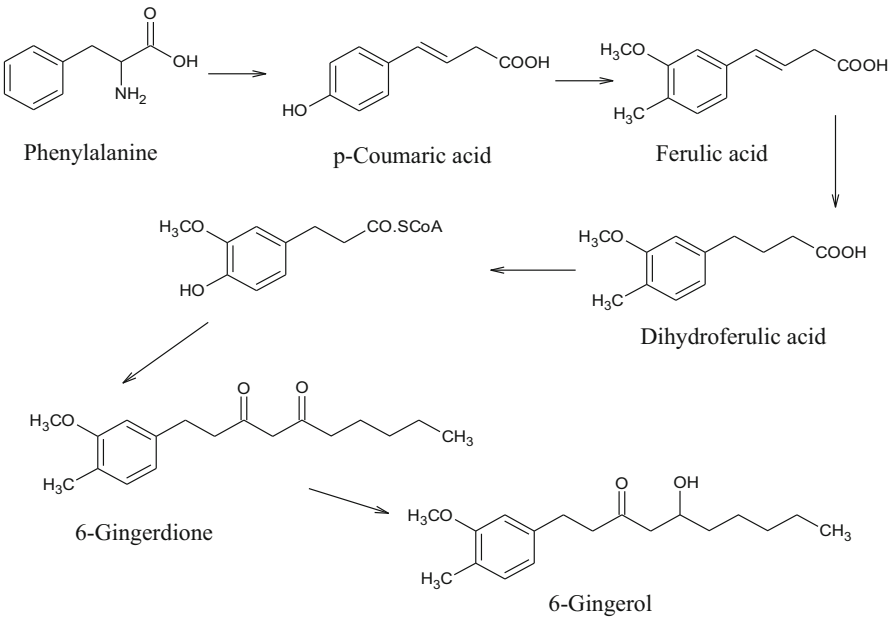


Fig. 2 Biosynthesis of 6-gingerol as proposed by Denniff et al. (1980)

galanolactone) have antiserotonin properties due to their antagonistic effect on the 5-HT3 receptors. The ability of these compounds to cross the blood-brain barrier might be achieved due to their small molecular masses (Mishra et al. 2012). Similar molecular observations resulted from the research of Yamahara and co-workers (1989), who tested 6, 8-, and 10-gingerol on an isolated ileum of guinea pig and observed the ability of all compounds to block the 5-HT3 receptors. In another study conducted on the house musk shrew animals (*Suncus murinus*), it has been shown that ginger acetone (150 mg/kg p.o.) extract and 6-gingerol (25 mg/kg p.o.) administered 60 min prior to cyclophosphamide successfully prevented the tested animals from emesis (Yamahara et al. 1989a). Further studies of Yamahara and co-workers (1990) in mice revealed that ginger acetone extract (75 mg/kg) and its metabolites (6-gingerol at 2.5 mg/kg, 6-, 8-, and 10-gingerol at 5 mg/kg) supported the transport of charcoal meal travel along the digestive system, leading to a conclusion that ginger extracts and its metabolites have significantly increased the speed of gastric emptying and the intestinal transit. On the other hand, according to Ali et al. (2008), ginger phenolic compounds are capable of gastric motility reduction (they inhibit the development of slow-wave stomach cramps) and therefore act as antiemetics. These effects were observed in dogs and rats protected from cisplatin-evoked vomiting by 50% ethanolic extract rich in gingerols and shogaols.

In consideration to the above-described examples of research, ginger extracts and phenolic compounds thereof are capable to influence the motility of

gastrointestinal tract by influencing the muscle contraction and blocking the functions of serotoninergic receptors in the digestive system.

In traditional medicine, ginger has been recommended in the morning nausea occurring in pregnancy, especially in the first trimester, but caution is required when taking the drug. The daily dose of powdered ginger should not exceed 2 g (Grys et al. 2010). Studies on pregnant ginger-fed rats by Wilkinson (2000) showed that their fetuses have higher weight and a better developed skeleton; however, the miscarriages may occur more often among these animals.

37.4.2 Antibacterial, Antifungal, and Anthelmintic Effects

The antimicrobial properties of ginger have been widely studied. Its water-ethanol extract was found to possess antibacterial properties against Gram-positive and Gram-negative (Chrubasik et al. 2005) bacteria, which is quite rare for the plant extracts. Several strains susceptible to ginger polar extracts were described by different authors and included the data on the inhibitory properties of ginger against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Escherichia coli* (Ali et al. 2008). Boiled ginger rhizomes were found effective against *Escherichia coli*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Mycobacterium phlei*, *Streptococcus faecalis*, and *Bacillus cereus* (Chrubasik et al. 2005). Unfortunately gingerols and shogaols have not yet been enough investigated for their antimicrobial properties so far.

In one study the methanolic extract of raw ginger powder and the isolated compounds 6-, 8-, and 10-gingerol and 6-shogaol inhibited the growth of some *Helicobacter pylori* strains. Still, in this case, the total extract was found more effective in comparison with the isolates, whose MIC's ranged from 1.6 to 25 μg/mL with 10-gingerol as the strongest component (Mahady et al. 2003). According to the study of Park and co-investigators (2008), the derivatives of gingerol (10- and 12-gingerol) inhibited the growth of oral bacteria: the pathogens like *Porphyromonas gingivalis*, *P. endodontalis*, *and Prevotella intermedia* with the MIC values calculated from 6 to 30 μg/mL. 10-Gingerol was found active against *Bacillus cereus* (MIC = 250 ppm) (Cetin-Karaca and Newman 2018). Also, the growth of two strains of *Mycobacterium*, *M. avium* and *M. tuberculosis*, was restricted by 6-gingerol (Semwal et al. 2015).

The phenolic compounds from ginger were found to increase the activity of some antibiotics, like aminoglycosides, against vancomycin-resistant strains of *Enterococcus*. For *E. faecalis* the MIC of an antibiotic arbekacin was decreased from 64 to 4 μg/mL, when joined with 10-gingerol (Semwal et al. 2015).

Only a few studies report the inhibitory properties of ginger against fungi. Hydroalcoholic extracts of *Zingiber officinale* inhibited the growth of *Candida albicans* in the study of Jagetia and co-investigators (2003). Another study performed by Ficker and co-workers (2003) on the African ginger phenolics showed that three gingerols and gingerdiol exhibited the antifungal effect against 13 strains

at the concentration lower than 1 mg/mL. 8-Gingerol was found to be more active from 6-gingerol and gingerdiol according to this study. Even if these compounds exhibited antifungal potential, still their activity was calculated as more than 10 times weaker from the antifungal drug: amphotericin B.

37.4.3 Anthelmintic Properties

Anthelmintic activity of ginger extracts was tested on several species of parasites. Its antiparasitic properties were confirmed, e.g., against *Angiostrongylus cantonensis*, *Anisakis simplex* larvae present in fish, *Aedes aegypti*, *Schistosoma mansoni* (Aly and Mantawy 2013), and *Culex quinquefasciatus* (Lin et al. 2010; Ali et al. 2008).

Raw, aqueous ginger extract was administered to sheep infected with nematodes, and depending on the dose of ginger and the duration of treatment, anthelmintic activity was noted (Ali et al. 2008).

The studies on single metabolites of ginger are less abundant. In the scientific paper of Lin and co-investigators (2014) among 11 single constituents obtained from ginger extracts, the cestocidal activity and/or the ability to inhibit the movements of *Hymenolepis nana*, 10-shogaol, and 10-gingerol exhibited the biggest potential in a dose- and time-dependent manner. The parasite exposed to the action of the former compound at the concentration of 200 μM had died within 12 h. Similar results were presented in case of *Anisakis simplex*. The maximum lethal efficiency and larvicidal action were obtained for both 10-shogaol and 10-gingerol; however, in this case the latter compound was found preferable and stronger (Lin et al. 2010).

37.4.4 Effects on the Digestive System

Powdered rhizome ginger has a long history of use in traditional medicine as a remedy alleviating the symptoms of diseases of the digestive system. Acetone extract from ginger improves gastric motility and has a diastolic effect on the smooth muscle of the gastrointestinal tract, so it can be used to relieve indigestion, bloating, and general symptoms of irritable bowel syndrome (Ali et al. 2008). Among the metabolites of ginger, according to Yamahara and collaborators, 6-shogaol gave a more visible anticathartic effect than 8- and 10-gingerol and 6-dehydrogingerdione. 6-, 8-, and 10-gingerol and 6-shogol administered orally enhanced the transport across the digestive system and the emptying of the stomach, in a similar manner than that of metoclopramide (Chrubasik et al. 2005).

The protective role of ginger compounds has been demonstrated to inhibit the development of *Helicobacter pylori* in vitro, which is responsible for indigestion, stomach ulcers, and the development of colorectal cancer (Ali et al. 2008; Mishra et al. 2012).

In contrast to oral administration, intravenous injection of 6-shogaol inhibited the transport through the digestive tract. According to these studies, 6-gingerol and

10-gingerol caused an increase in bile secretion and increased the activity of pancreatic and intestinal lipases (Chrubasik et al. 2005).

In addition, ginger enhanced the appetite, eliminated the feeling of fullness after eating fatty foods, and protected the animals against the formation of ulcers after ingestion of alcohol or drugs (Ali et al. 2008). The rhizomes were found to possess a protective effect on the mucous layer of the stomach, by increasing its resistance to harmful factors (including alcohol, acetylsalicylic acid, and other drugs) (Shukla and Singh 2007). In the studies of Weng and collaborators, an oral dose of 1.5–50 mg/kg of 6-gingerol inhibited the secretion of basal acid, also in conjunction with capsaicin (Semwal et al. 2015).

Oral administration of unpolar constituents of ginger (e.g., zingiberene) and 6-gingerol (at a dose of 100 mg/kg) inhibited the occurrence of gastric lesions in mice by 54%, each, according to the study of Yamahara cited by Chrubasik and co-workers (2005). This particular activity was strictly correlated with the prevention of gastric ulcer at a similar level to nonsteroidal anti-inflammatory drugs.

Furthermore, 6-shogaol and some ginger terpenes (*ar*-curcumene, *b*-sesquiphellandrene, *b*-bisabolene) (Yamahara and co-investigators), 6-gingesulfonic acid, and 6-gingerol (Yoshikawa and co-workers) were found to exhibit anti-ulcer properties in rats. Interestingly, the process of rhizome roasting enhanced the protective activity (an active dose of 170.6 g/kg).

Ginger extract, 6-gingerol and 10-gingerol, administered in rats exhibited cholagogue properties (Yamahara and co-investigators) and, at a dose of 50 mg% within 8 weeks, an enhanced pancreatic lipase activity (Platel and Srinivasan) (Chrubasik et al. 2005). Also, its effect on the stimulation of pancreatic lipase, amylase, and proteinases (chymotrypsin, trypsin, carboxypeptidase) was described in rats (Srinivasan 2017).

6-Gingerol was responsible for the lowering of the total bilirubin content and hepatic marker enzymes (phosphatase, aminotransferases) in a similar manner as silymarin, which was described in the studies of Sabina and collaborators (Semwal et al. 2015). In the studies on rats, both 6-gingerol and 6-shogaol protected liver from the toxic effects of diclofenac at a dose of 10 mg/kg (i.p.), when administered for 6 days (studies of Alquasoumi and collaborators) (Semwal et al. 2015).

37.4.5 Effects on the Cardiovascular System

Ginger extracts and its constituents due to their highly described anti-inflammatory, antioxidant, hypotensive, antiplatelet, and hypolipidemic effects supported by the results of numerous in vivo studies are perceived to play a significant protective role in cardiovascular diseases.

In the conducted studies on rats, the indirect and direct influence of ginger components on the value of arterial blood pressure and heart rate was suggested (Mishra et al. 2012). The studies describe the effect of raw ginger extract on lowering arterial blood pressure in anesthetized rats and reducing heart rate and contractility, heart rate reduction, and spontaneous contraction force in guinea pigs. The ginger pressure-lowering effect can be caused indirectly by blocking voltage-dependent

calcium channels (Nicoll and Henein 2007). Interestingly, it has been noticed that some active ingredients of ginger may have opposite properties (Chrubasik et al. 2005; Ali et al. 2008). In smaller doses these compounds possess cardio-depressant properties, whereas at higher doses, the cardiotonic properties were observed (Mishra et al. 2012). 8-Gingerol in the studies of Kobayashi and co-workers at the concentrations of $1 \times 10–6$ to $3 \times 10–5$ M resulted in a positive inotropic effect on a left atrium of guinea pig (Semwal et al. 2015).

According to the Suekawa and collaborators, who studied the effects of single ginger compounds, likewise to capsaicin, 6-shogaol caused a rapid decrease in the blood pressure and induced bradycardia in rats. This particular activity was reversed by the administration of calcium antagonists and α-adrenoreceptor blockage. Also, 6-gingerol at lower doses evoked the response of the depressor on the blood pressure, when injected to the bloodstream (Chrubasik et al. 2005). In the study of Liu and co-workers, 6-gingerol was proven to be an inhibitor of the angiotensin II type 1 receptor with IC50 value of 8.2 μM (IC50 of angiotensin = 1 μM) and could be therefore treated as a blood pressure regulator. Other studies confirm its additional anti-atherosclerotic activity related to the inhibition of the incorporation of [35S]-Met/Cys into the vascular proteoglycans, leading to a decreased protein synthesis and protein synthesis (Semwal et al. 2015).

Gingerols and especially 6-gingerol were able to increase the thermogenesis in animal studies in a stronger manner than the respective shogaols. Considering this, the authors of the study (Eldershaw and collaborators) observed vasoconstriction effects. Another manuscript described the occurrence of an increased intestinal blood flow with no effect on the arterial blood pressure (assessed by Doppler flowmetry) upon the intraduodenal administration of dried ginger with high concentration (2 mg/kg) of 6-shogaol (Chrubasik et al. 2005), which could support an increased digestion upon the ginger intake.

6-Gingerol, 6-shogaol, and gingerdiones were reported to possess inhibitory properties against thromboxane, leukotriene, and prostaglandin biosynthesis (Mishra et al. 2012). 6-Gingerol administered at a dose of 10 mg/kg ameliorated the elevation heart enzymes induced by doxorubicin in rats, such as NF-KB, sRAGE, and cardiac caspase-3, so it may be helpful as a cardioprotecting agent (Semwal et al. 2015). In fact, the cardioprotective properties of ginger were studied also concerning its eventual prevention of myocardial infarction. In Wistar rats pretreated with ginger extract for 4 weeks prior to the induced infarction, significantly lower levels of cardiac markers were noted. Those included alanine transaminase, lactate dehydrogenase, aspartate transaminase, and creatine kinase-MB isoenzyme. Furthermore, an improved concentration of antioxidant enzymes was seen for those pretreated rats in relation to a control group (Srinivasan 2017).

37.4.6 Impact on the Metabolism of Lipids

Treatment with methanolic extract from the dried ginger rhizome resulted in a significant reduction of the level of triglycerides, phospholipids, and LDL cholesterol resulting in the decrease of body weight and decreased levels of

hypercholesterolemia, hyperglycemia, and hyperinsulinemia, in animal models. A reduction in urinary protein and a decrease in urine output were also observed. In mice deficient in apolipoprotein E after ginger supplementation, the LDL oxidation was inhibited, and the development of atherosclerosis was slowed down (Mishra et al. 2012) in a similar manner to the activity of ascorbic acid (Ahmed et al. 2000). Moreover, ginger reduced the synthesis of cholesterol in the liver and had an accelerating effect on its conversion to bile acids, their excretion in the feces (Nicoll and Henein 2007), and activation of pancreatic lipase (Srinivasan 2017). In the studies on rats fed with 0.5% ginger oleoresin for 20 days, fecal cholesterol levels were higher compared to the control group, together with a decreased serum and liver levels of cholesterol (Srinivasan 2017).

According to the studies, the metabolite responsible for the above listed properties in the highest extent is 6-gingerol as it is involved in the metabolism of lipids (Ghosh et al. 2011).

In the studies of Okamoto and collaborators, 6-gingerol reduced fat accumulation in mice as, according to Tzeng and Liu, it was able to suppress the accumulation of oil droplets by decreasing their size in the 3 T3-L1 cells (Semwal et al. 2015). The anti-obesogenic properties evaluated in various in vivo studies were related to the stimulation of PPAR δ (peroxisome proliferator-activated receptor δ), to the regulation of PPARγ pathway in adipocytes, and to the inhibition of inflammatory markers, by ginger and its metabolites. These properties were visible in a catabolic action toward lipids by 6-gingerol and 6-shogaol observed without high lipid food intake being affected (Srinivasan 2017).

Also, a significant decrease in obesity, glucose, and insulin levels was described in diabetic rats, where ginger treatment (mainly due to the presence of 6-gingerol) lowered serum glucose, cholesterol, and triglycerides' levels (Ali et al. 2008; Semwal et al. 2015). Its mechanism of action could be related to the ability to inhibit the HMG-CoA reductase and the subsequent activation of LDL receptors in diabetic rats. The administration of ginger ethanolic extract at a dose of 800 mg/kg noticeably decreased the blood sugar level after 1 h, with a maximum effect recorded after 4 h (Srinivasan 2017). An enhanced glucose uptake was proven to be related to the presence of 6- and 8-gingerols as demonstrated by Li and collaborators upon the analysis of L6 myotubes. Stronger activity was attributed to 8-gingerol who stimulated an increased surface distribution of the GLUT4 protein in the myotube membranes (Semwal et al. 2015). Antidiabetic properties were also observed in diabetic rodents through an increase in insulin sensitivity and the protective effects toward β-pancreatic cells reflected in a lowered antioxidative stress (6-gingerol stimulated catalase, GSH, superoxide dismutase, and glutathione peroxidase), higher glucose uptake by the peripheral cells, and reduced accumulation of fat. Gingerols and shogaols treatment (administered in the form of polar extracts rich in phenolic metabolites) appeared to reconstruct the kidney tubules by their antiglycation activity, to reduce the fatty infiltrations, and to inhibit diabetes-related formation of cataract (Srinivasan 2017; Semwal et al. 2015).

37.4.7 Anti-inflammatory, Anticoagulant, and Analgesic Properties

Ginger has long been known as an anti-inflammatory, anticoagulant, and analgesic agent (Ross 2005). It inhibits prostaglandin biosynthesis, disrupts the arachidonic acid cascade, and blocks vanilloid receptors (associated with the chemical and thermal nociceptive stimuli) (Ali et al. 2008).

It has similar properties to nonsteroidal anti-inflammatory drugs (NSAIDs) because it suppresses prostaglandin synthesis by inhibiting the COX-2 enzyme (cyclooxygenase-2) and leukotriene biosynthesis by affecting the 5-lipoxygenase enzyme (LOX-5) (Ali et al. 2008). According to some various studies, it does not inhibit COX-1 (Cisowski et al. 2004; van Breemen et al. 2011). Thanks to these properties, ginger has a better therapeutic profile and fewer side effects than drugs belonging to the NSAID group.

The extract obtained from *Zingiber officinale* was proven to inhibit the activity of several genes involved in the formation of inflammatory response, as well as genes encoding cytokines and chemokines and inducing the COX-2 enzyme (Grzanna et al. 2005; Ali et al. 2008).

Attention is also paid to the effect of ginger and its compounds on the transcription factors that regulate the expression of genes responsible for inflammation. These factors include NF-κB and AP-1, which under the influence of, for example, lipopolysaccharides from G (−) bacteria lead to an increased expression of genes and proteins for TNF-α, IL-1, IL-12, iNOS, and COX-2 (Lantz et al. 2007).

In one of the studies performed on rats, an oral or intraperitoneal intake of ginger extract at a dose of 50 mg/kg triggered a statistically significant reduction in serum PGE 2 levels. Higher dose of 500 mg/kg administered orally reduced TXB2 level, and the same dose administered orally and intraperitoneally decreased the level of PGE2. In addition, when using a higher dose of ginger, a reduction in serum cholesterol was observed (Ali et al. 2008). Other experiments indicate that ethanolic ginger extract reduces rat swelling caused by carrageenan and also reduces yeast-induced fever (Ali et al. 2008).

In the presence of phenolic compounds with a long side alkyl chain such as [6]-gingerol, [6]-shogaol, [4]-shogaol, or [12]-shogaol, stronger inhibitory properties were observed toward the synthesis of leukotrienes than toward prostaglandins as proven for ginger extracts. The length of the chain also affected the efficiency of COX-2 inhibition in the study of Kiuchi and collaborators. The compounds lower than 8-gingerol, 8-shogaol, and 8-paradol were less active from the remaining components with a longer chain. Higher than 10-gingerol and its derivatives, a plateau in the pharmacological potential was observed (Kiuchi et al. 1992).

Ginger extract containing a mixture of compounds 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol showed in subsequent tests more inhibitory activity against PGE2 than each of them separately. The obtained results allowed to arrange phenolic compounds in terms of their ability to inhibit the production of PGE2 in the following order: 10-gingerol <8-gingerol <6-gingerol <6-shogaol. These phenolic compounds not only inhibited COX-2 enzyme activity but also reduced the level of

COX-2 mRNA (Lantz et al. 2007). In terms of a low toxicity ratio of ginger metabolites toward the digestive system, they were found preferable from the NSAIDs (Ali et al. 2008). Besides the above-described effects, ginger metabolites were also found to inhibit the antigen presentation (6-gingerol), the NOS, IL-1β, IL-6, NO synthase and TNF-α expression (by suppressing the 1-κBα phosphorylation and PKC-α translocation), NF-κB activation by inhibiting intracellular adhesion molecule-1 (10-gingerol, 6- and 8-shogaol), and the radical processes (Semwal et al. 2015). Also, some in vivo models on animals were applied to determine ginger's anti-inflammatory potential. According to these tests, 6-gingerol was proven to block the paw edema effects at a dose of 100 mg/kg in mice, to inhibit the iNOS and COX-2 protein expression, and to influence the transcriptional properties of NF-κB by phosphorylation blockage (Semwal et al. 2015). A possible anti-inflammatory mechanism of ginger's activity may be related to its affinity to calcium channels by stimulating its influx and an intracellular release of calcium. According to different studies, shogaols are more potent than gingerols, and they were successfully used in the treatment of arthritis or swelling (Bode and Dong 2011).

Ginger extracts were proven to exhibit **anticoagulant** properties due to the inhibition of platelet aggregation and thromboxane-B2 production in the animal studies on rats (Srinivasan 2017; Thomson et al. 2002; Ali et al. 2008). Ginger metabolites were suggested as new inhibitors of platelet activation, with no side effects of acetylsalicylic acid (Bode and Dong 2011). These properties could be affected by the presence of 6-gingerol in the extract. According to the studies of Guh and collaborators, the release of arachidonic acid cascade was inhibited by 6-gingerol at the concentrations of 0.5–20 μM whereas the inhibition of thromboxane B2 formation at a dose of 0.5–10 μM (Semwal et al. 2015). Even if some results of this particular pharmacological activity have been published, the anticoagulant properties of ginger are still doubtful due to a number of tests on human with no effect observed (see Sect. 5).

6-Shogaol was proven to deliver **antinociceptive** properties and was perceived as a substance P inhibitor in the tests on rats (Mishra et al. 2012).

37.4.8 Antioxidant Properties

Ginger is one of the stronger natural antioxidants. The rhizomes themselves, as well as extracts obtained from them, contain a significant amount of polyphenolic compounds. The activity of ginger and its metabolites is based not only on the radical scavenging properties but also on the stimulation of natural antioxidant enzymes, on the inhibition of inflammatory processes related to the arachidonate metabolism, on the inhibition of lipids' oxidation, and on the inhibition of nitric oxide synthase (Srinivasan 2017).

Recently, numerous tests were carried out to determine the scavenging power of ginger metabolites against free radicals, which include the reactions with DPPH reagent, linoleic acid, Fe3+ ions, xanthine oxidase, and others (Peng et al. 2012; Stoilova et al. 2007). In in vitro (butylated hydroxytoluene), ginger extract showed a

similar antioxidant strength (Stoilova et al. 2007). Most often its potential was studied spectrophotometrically, in reaction mixtures with radicals or in cell homogenates (e.g., the studies on the ability to chelate iron (III) ions, inhibit xanthine oxidase, inactivate H_2O_2 and lipid peroxidation in rat brain homogenates) (Peng et al. 2012; Nicoll and Henein 2007). Studies performed on single metabolites proved a marked potential of 6-shogaol, which was found to be the strongest component (IC50 = 8.05 μM), possibly to the presence of α, β-unsaturated ketones in its structure, opposed to the weakest scavenger: 6-gingerol (IC50 = 27 μM). 10-gingerol with a long carbon chain was found effective, too (Dugasania et al. 2010). These properties in turn may lead to a reduction or inhibition of damage caused by radicals in cell membranes and in DNA (Stoilova et al. 2007; Dugasania et al. 2010).

Studies in rats have demonstrated a significant effect of ginger on the reduction of lipid peroxidation and an increase in serum antioxidant and glutathione levels (Nicoll and Henein 2007), which was helpful in the cardiac disorders. On the other hand, diabetic animals were also benefiting from these particular properties of ginger metabolites. In a study on diabetic rodents, Akash and collaborators denoted protective effects toward the B cells in the pancreas, Ahmadi et al. described the antioxidant properties impeding glycation upon the administration of 200 mg/kg ginger extract for 30 days in diabetic rats, and Shanmugam et al. described a neuroprotective effect caused by the acceleration of antioxidant mechanisms in the brain. The latter properties were visible in the hippocampus, hypothalamus, and cerebellum of diabetic rats, which also confirms a satisfactory permeability of ginger phenols through the brain-blood barrier (Srinivasan 2017). Even if some effects of ginger antioxidant activity are described, detailed mechanisms and specific targets are still missing in the scientific reports.

37.4.9 Effect on Cancer Prevention

The anticancer activity assessment of both ginger extracts and ginger metabolites has been widely discussed and published. The researchers underline a marked anticancer activity of the extract itself but also of single metabolites, like zingerone, 6-gingerol, and 6-paradol in their tests. The authors mention their proapoptic properties related to mitochondrial and the bcl-2 family mediation (increase in the Bax/Bcl-2 ratio), by the disturbances in the cell cycle progression (cell cycle arrest at the G2/M phase with only little impact on the sub-G1 phase), and disturbed reproductive capacity with no influence on the healthy host cells (Srinivasan 2017; Lima et al. 2018).

The anticancer properties of 6-gingerol and zerumbone were associated with their high antioxidant capacity, which played a crucial role in the reduction of peroxide levels (Bode and Dong 2011). As mentioned in the subsection on the anti-inflammatory properties of ginger, its compounds exhibit a downregulating effect on the transcription factors and signalling molecules (NF-κB, TNF-α, COX-1, Bcl-2, Mcl-1, AP-1 survivin, cyclin D1, CDS-4, proto-oncogene proteins – c-Myc, hTERT, and caspases), which influence the formation of tumors

(Srinivasan 2017; Lima et al. 2018). Among caspases influenced by the presence of ginger extracts or their metabolites, caspase-3 and caspase-9 are the most frequently indicated. Also, ginger administration led to an upregulated IκB-α and protein p21 level. The inhibition of proto-oncogene proteins like previously mentioned c-Myc or hTERT speaks for the specificity of ginger extracts against certain types of tumors, e.g., breast cancer (Lima et al. 2018).

Ginger metabolites were proven to directly inhibit the AP-1 DNA-binding activity (6-gingerol) or to directly induce apoptosis (6-paradol) as described by Bode and co-workers (2011). According to Lin and collaborators, the proapoptic properties of 6-gingerol could be related to the enhanced levels of negative cell cycle regulators (p21Cip1 and p27Kip1), phosphorylation of p53 protein, and enhanced ROS levels inside the tumor (Lima et al. 2018). Gingerol is perceived as a tumor necrosis sensitizing drug, as it clearly induces a ligand (TRAIL)-mediated apoptosis, e.g., in glioblastoma cells, being normally resistant to TRAIL signalling. These properties were observed based on the elevated levels of survivin, Bcl-2, XIAP (X-linked chromosome to apoptosis inhibitor), or cFLIP. According to Lee and coinvestigators, these compounds could be administered in patients with TRAIL-resistant glioblastoma incidence (Lima et al 2018). Rastogi et al. underlined that the triggered generation of ROS in the tumor by ginger metabolites inhibited the mitochondrial inhibitory complex I and led to the induction of cell death by an increased miR-27b expression and the following DNA damage (Lima et al. 2018).

Interestingly, synergistic effects of action were observed upon the administration of ginger total extract with an isolated 6-gingerol. In the studies of Brahmbhatt and co-workers, the combination resulted in a marked increase in the antiproliferative effect of ginger extract (Srinivasan 2017). 10-Gingerol was proven to increase an intracellular calcium level and induce cytotoxic effects in colorectal cancer cell lines (Bode and Dong 2011).

Another interesting remark was published by the research group of Cheng et al. (2011), who discovered differences in the antiproliferative effect of cooked ginger in comparison with the unprocessed one. According to the researchers, the anticancer potential of the former was about twice as high as of fresh and dried ginger rhizomes. Chromatographic analysis confirmed that a 4-h-long boiling at 120 °C changed the composition of the extracts toward a higher content of shogaols in relation to gingerols.

Interestingly, in the studies performed on a cellular level, ginger phenols and ginger extract were found to decrease the toxicity of chemotherapeutics administered to patients with tumors. In a study of Hosseini et al., the cardiac toxicity of doxorubicin was reversed upon the administration of ginger capable to induce marked antioxidant potential. These properties were confirmed by the suppression of apoptosis and by the reduction in lipid peroxidation (Lima et al. 2018).

The reports are mainly based on the in vitro studies on cell lines, as they offer fast protocols for the assessment of chemopreventive properties in various types of cancer. However, Table 3 lists the results of in vivo studies performed on animal models for a better clarity of the presented data.

Table 3 Selected anticancer studies of ginger and its metabolites

Material tested	Animal model	Type of cancer	Observed activity	Research group/ reference
6-Gingerol (6-GN)	Mice	Skin cancer	Topical application of 6-GN, 6-paradol, and 6-dehydroparadol suppressed the stimulated skin irritation and inflammation, TNF-α production	Surh et al., Chung et al./ Srinivasan 2017, almighty ginger Nigam et al./ Semwal et al. 2015
	Mice	Colon carcinoma 3 μg/mL p.o.	Increase in the infiltration of tumors by lymphocytes (CD4, CD8 T cells, B220 + B cells) and a reduction in the number of CD4 + Foxp3 + regulatory T cells	Ju et al./Semwal et al. 2015
	Mice	B16F1 (mouse skin melanoma)	Increase in the infiltration of tumors by lymphocytes (CD4, CD8 T cells, B220 + B cells) and a reduction in the number of CD4 + Foxp3 + regulatory T cells	Ju et al./Semwal et al. 2015
	Mice	Benzo[a] pyrene-induced skin tumor 2.5 μM/mouse	Reduced the number of tumors and their volume (increased p53 levels)	Nigam et al./ Semwal et al. 2015
	Mice	TPA-induced skin tumor 25 μM/mouse	Inhibition of COX-2 expression, suppression of NF-κB DNA-binding properties and of p38 phosphorylation	Kim et al./ Semwal et al. 2015
	Mice	Murine renal cell carcinoma Topical administration 1 and 2.5 μM	Increase in the infiltration of tumors by lymphocytes (CD4, CD8 T cells, B220 + B cells) and a reduction in the number of CD4 + Foxp3 + regulatory T cells	Ju et al./Semwal et al. 2015
	Mice	Prostate cancer 10 mg/kg p.o. 15 days	Modulation of proteins engaged in the apoptosis pathway	Shukla et al./ Semwal et al. 2015
	Male mice	Pancreatic cancer i.p.	Decreased tumor incidence	Akimoto et al./Li et al. 2018
	Nude mice	Tumor induction 2.5 and 5 mg/kg i.p. 45 days	Induction of apoptosis and upregulation of p53 protein	Rastogi et al./ Lima et al. 2018

(continued)

Table 3 (continued)

Material tested	Animal model	Type of cancer	Observed activity	Research group/ reference
Ginger extract	Nude mice	Prostate cancer 100 mg/kg p.o.	Inhibited progression of cancer by the perturbed cell cycle progression and proapoptic properties	Karna et al./ Srinivasan 2017
	Rats	Colon cancer 50 mg/kg	Suppressive activity against tumor by a decreased oxidative damage (enhanced superoxide dismutase, catalase, glutathione peroxidase, and transferase) and inhibition of β-glucuronidase and mucinase microbial enzymes	Yoshimi et al.; Manju and Nalini/almighty ginger
	Nude mice	Induced prostate cancer 100 mg/kg 8 weeks p.o.	Decreased Ki67, cyclin B, D, and E levels	Karna et al./Li et al. 2018
6-Shogaol	Xenograft mouse model	Colon cancer 15 mg/kg i.p.	Inhibition of HCT-116 (arrested at the G2/M phase by the regulation of p53/p21 pathway) and SW-480 cell proliferation (IC50 = 7.5 and 10 μM, respectively)	Qi et al./ Srinivasan 2017
	Mice	TPA-induced skin tumor 3 μM/mL, 60 days	Inhibition of TPA evoked iNOS and COX-2 mRNA expression (suppressed nuclear translocation of NF-κ B subunits)	Kim et al./ Semwal et al. 2015

37.4.10 Varia

Rodent tests confirmed a reduction in mortality of gamma-prone mice as well as the ability of ginger extracts to inhibit the **radiotherapy**-induced emesis. Therefore, ginger can reduce the damage that occurred during irradiation (Ali et al. 2008) and also because of these can be successfully used in sunscreens, due to its effectiveness in protecting the skin against UV-B radiation (Mishra et al. 2012).

Several **central nervous system** activities of ginger extract and single metabolites were described. Powdered ginger at a dose of 500–600 mg given early enough before the onset of migraine brings relief during its attack (Ghosh et al. 2011). Ginger has the ability to inhibit monoamine oxidase A (MAO-A). Its increased activity and expression of MAO mRNA occurs in people with Alzheimer's and depression. The MAO-A inhibition is indicative of neuroprotective and

antidepressant properties and slowing down the progression of Parkinson's disease (Peng et al. 2012).

Both terpenes and phenols were found to exhibit these properties; however, possibly a higher antidepressant potential was related to the presence of lipophilic components, as described in the study of Kukula-Koch and collaborators (2018).

The statistics show a significant effect of the ginger extract on the reduction of symptoms of knee degenerative diseases (Ghosh et al. 2011). Ginger extracts were found promising in the rheumatoid arthritis, edema, and inflammation, as shown in animal studies (Mishra et al. 2012). In some animal tests, ginger compounds were proven to milden the effects of induced rheumatoid arthritis and streptococcal cell wall-induced arthritis. Funk and collaborators concluded that dichloromethane extract rich in unpolar compounds was more effective from the one rich in phenolic fractions, mainly 6-gingerol (Mishra et al. 2012).

The hepatoprotective effects of ethanolic extracts administered orally were confirmed in the studies on rats with CCl_4- and paracetamol-induced hepatotoxicity described by Ezenou et al. and Abdullah et al. The levels of superoxide dismutase (SOD), malondialdehyde (MDA), and aspartic transaminase (AST) were significantly reduced upon the administration of 200 mg/kg (for SOD) and 300 mg/kg (for the remaining levels) of extract. Also serum levels of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were influenced but at higher doses of extract, namely, 1000 mg/kg.

6-Gingerol was proven to exhibit an **immune-stimulating** effect. It is believed that it eliminates cough, has a warming effect on the body, and protects against small infections of the respiratory system (Mishra et al. 2012).

Oral and intravenous administration of 6-gingerol and 6-shogaol at the doses of 1.8–3.5 mg/kg for the former compound and 70–140 mg/kg for the latter showed visible **analgesic** and **antipyretic** effects; however, the researchers noted its cumulative sleep-inducing effect with hexobarbital. Similarly, in the studies of Young and collaborators, 6-gingerol administered in the form of intraperitoneal injection at the dosage of 25 and 50 mg/kg showed analgesic properties (Semwal et al. 2015).

8-Gingerol inhibited **melanogenesis** in the assays on mouse skin cells through downregulation of the MAPK and PKA signal pathways. Therefore, this compound may be used as a skin whitening agent (Huang et al. 2013).

37.5 Benefits (Human Studies)

Zingiber officinale belongs to the most studied herbal drugs. A variety of clinical studies performed on the groups of patients or volunteers are available in the scientific literature. This chapter intends to collect some selected ones, which deliver crucial information on the applicability of this plant in health disorders in which ginger is the most frequently used.

37.5.1 Influence on the Digestive System

37.5.1.1 Antiemetic Properties

The highest quantity of clinical studies was found for these particular properties. Among them there are voices confirming the antiemetic activity of ginger, but also those, which negate these findings. Nevertheless, the conducted studies were mainly considering the groups of pregnant women, as nausea and vomiting affect up to 80% of females at their first trimester of pregnancy (Bodagh et al. 2019). Clinical trials proved that ginger at a dose of 1–2 g per day is effective in pregnancy vomiting or nausea.

Other studies described the postoperative nausea in both sexes, which occurs in 30–70% of patients within 24 h after the operation. The administration of ginger at similar doses to the previously described protocols results in the lesser severity of mild stomach discomfort or diarrhea (Ball et al. 2015).

Also the behavior of tumor patients during chemotherapy was studied. Nausea is often evoked by cisplatin or cyclophosphamide, which is normally limited by serotonin receptor (5-HT3) inhibitors and glucocorticoids (e.g., dexamethasone). These drugs are bearing serious side effects, and their activity is often not sufficient enough, which gives way to the ginger trials. Various researchers describe positive effect of ginger therapy rather in acute than delayed nausea and after 6 h from the administration (Bodagh et al. 2019).

Ernst and Pittler (2000) in their review set together the outcomes of different clinical trials describing the influence of ginger on nausea and vomiting. Two out of three examples concerning postoperative nausea and vomiting confirmed a noticeable antiemetic activity of ginger in relation to placebo and its equal strength as metoclopramide. Divergent results leave this activity still debatable (1 g of ginger taken before operation obtained an absolute risk reduction of 0.052, which includes the possibility of no profit).

Other, newer and better planned trials (double-blind, well-controlled, randomized tests) confirm the influence of ginger on emesis in 30 hospitalized women before their 20 weeks gestational age. The patients were given 1 g of ginger or placebo every 4 days, followed by a 2-day washout before crossover. As a result 70% of group members responded positively to the treatment (placebo response: 15% only) with a statistical significance of $p = 0.035$ and a mean relief score of 3.7 to 4.1 (placebo: 0.1 to 0.9). Among the study outcomes, the patients described a reduced nausea and more rare incidence of vomiting (Bryer 2005). Smith and co-investigators intended to compare the strength of ginger with vitamin B6 in treating vomiting and nausea in 291 outpatient women at the beginning of their pregnancy (before 16th week of gestational age). The women were self-administered with 25 mg of vitamin B6 and 350 mg of ginger, three times a day and for 3 following weeks. In consequence both drugs were perceived as similarly effective in reducing vomiting (mean difference of 0.5), nausea (of 0.2), and dry retching (of 0.3). Neither of drugs had influence on the pregnancy course (Bryer 2005).

The antiemetic properties of ginger in motion sickness were demonstrated by testing 36 volunteers aged about 19 years who were given ginger and induced nausea

using a rotating chair. A reduction in the frequency of vomiting and nausea was observed after the administration of ginger (Chrubasik et al. 2005; Grys et al. 2010).

Malfunctions in the gastric emptying contribute to gastrointestinal problems, like nausea or dyspepsia. Their symptoms are often related to the feeling of fullness, epigastric burning, early satiety, discomfort in the upper abdomen, or gastroesophageal reflux, which frequently appear in correlation with meals (Bodagh et al. 2019). Several clinical trials studied the effect of ginger rhizome administered together with artichoke on dyspepsia and gastric emptying. Lazzini and colleagues determined the gastric volume by ultrasonography – before and after the meal. They proved that the administered herbs helped the patients by increasing the volume of 24%, compared with placebo. These effects, according to Giacosa et al., might have been influenced by the ability of ginger and its phenolic constituents (6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol) to stimulate the cholinergic M3 and serotoninergic 5-HT3/5-HT4 receptors (Bodagh et al. 2019).

To elucidate the mechanism of ginger's action, Micklefield et al. in their trial on a group of 16 healthy volunteers who took 1 g of ginger per day, orally, proved that the rhizome had no effect on the emptying of the stomach, as it was previously believed, but increased antral motility in the phase III of the migrating motor complex (MMC). As a result of this action, an increased motor response of the body was noted with no effect on duodenal contractions (Mishra et al. 2012) (see Table 4).

37.5.2 Analgesic and Anti-inflammatory Properties

In humans, one study of Bliddal and colleagues did not provide significant differences between ginger and placebo in the group of osteoarthritis of the knee or hip. On the other hand, the study of Altman and Marcussen delivered suitable data to confirm a greater response from the knee osteoarthritis patients in comparison with placebo (Bode and Dong 2011). In another double-blind randomized study on 67 healthy adults in a postsurgical pain model described by Rayati and colleagues (2017), ginger anti-inflammatory and analgesic properties were of similar intensity as those of ibuprofen.

The researchers confirm the analgesic properties of ginger in various clinical trials. A relief from swelling and pain was reported by Ozgoli and collaborators in the trial on the patients with osteoarthritis, rheumatoid arthritis, or other muscular discomfort upon the administration of 250-mg capsules from powdered ginger for the period of 3 months to 2 years. The results of this double-blind clinical trial showed a similar strength of ginger as of ibuprofen administered at the quantity of 400 mg or mefenamic acid (250 mg) (Srivastava and Mustava).

The anti-inflammatory properties of ginger were not confirmed in a study of Black and Oconnor, who administered 2 g of ginger to patients 30 min prior to cycling exercise. As a result, the studied group did deliver quadriceps muscle pain, higher work rate, and heart rate (Bode and Dong 2011). However, Black et al., who administered 2 g of ginger for 11 days on a group of 36 participants, to cure muscle pain, proved moderate-to-large reductions (Mashhadi et al. 2013).

Table 4 Selected examples of clinical trials lead in the direction of anti-nausea and anti-vomiting properties of ginger

	Study group	Dosage	Reference	Comments	Reference
Vomiting and nausea	291 outpatient women <16 weeks' gestational age	350 mg of ginger rhizome 25 mg of vitamin B6 3 times a day 3 weeks	Ginger versus vitamin B6	Ginger was equivalent to vitamin B6 in nausea, vomiting, and dry retching reduction	Smith et al./in Bryer 2005
	26 outpatient women 7–11 weeks' gestational age	250 mg ginger syrup 4 times a day 1 week	Double-blind, randomized study: ginger versus placebo	67% patients stopped vomiting and nausea	Keating and Chez/in Bryer 2005
	70 outpatient pregnant women	1 g of ginger for 4 days	Double-blind randomized study Ginger versus placebo	Milder nausea in ginger group ($p = 0.014$) Reduction of 62% for ginger and 34% for placebo group	Vutayavanich et al./in Bryer 2005
	30 pregnant women	1 g of ginger 1 time a day for 4 days, followed by a 2-day washout	Double-blind randomized crossover study with 2-day washout before crossover Ginger versus placebo	Significant preference for ginger (70%) versus placebo (15%), $p = 0.035$ Reduced incidence of vomiting and decreased nausea	Fischer-Rasmussen et al./in Bryer 2005
	16 volunteers	1 g of ginger orally	–	Increased antral motility during phase III of the MMC – migrating motor complex	Micklefield et al./in Mishra et al. 2012
	120 women after gynecological surgery	1 g of powdered ginger 1 h prior to anesthesia	Ginger versus placebo or metoclopramide (10 mg)	Incidence of nausea: 21% ginger, 27% metoclopramide, 41% placebo, no side effects observed	Phillips et al./ in Mishra et al. 2012
	80 cadets	1 g of powdered ginger, 1 dose	Placebo 2 parallel groups	Significant relief after 4 h past administration, superior to placebo	Grontved/in Mishra et al. 2012
	34 patients during chemotherapy	500 mg Twice a day Orally 5 days	Placebo and crossover with antiemetics	No significant differences between the placebo and ginger	Thamlikitkul et al. (2017)/ in Bodagh et al. 2019

(continued)

Table 4 (continued)

	Study group	Dosage	Reference	Comments	Reference
	36 patients during chemotherapy	0.5 or 1.5 g of extract a day + antiemetic drug Consumption 2 × 6 day, evaluation 3 × 4 day	Placebo + antiemetic drug	Reduced incidence of acute but not delayed nausea in chemotherapy with cisplatin	Fahimi et al./ in Bodagh et al. 2019

To conclude, even if the results confirming the efficacy of using ginger preparations as anti-inflammatory and nociceptive agents are not unified, the positive voices still seem encouraging.

37.5.3 Other Activities

The results obtained from animal tests were confirmed in several human trials. In a study of Verma and colleagues, 5 g of ginger consumed by the studied group **inhibited the platelet aggregation** and, according to Verma and Bordia, induced fibrinolytic activity. In the studies on human blood cells among several single ginger constituents, 8-gingerol, 8-shogaol, and 8-paradol, the latter exhibited the strongest antiplatelet properties expressed as COX-1 inhibition with an IC50 value of ca 20 μM (Bode and Dong 2011).

On the other hand, ex vivo studies by Janssen and co-workers gave no effect of ginger oral consumption on thromboxane B2 levels on human blood samples (Srinivasan 2017).

The **antioxidant potential** of *Zingiber officinale* extract and 6-gingerol was confirmed in a clinical trial with the presence of 43 patients diagnosed with cancer. In this trial 19 patients were given 2 g of standardized ginger extract with an addition of 5 mg 6-gingerol a day and 24 placebo for a period of 3 consecutive days before the first cycle and during chemotherapy until the fourth cycle. According to Danwilai and colleagues, increased level of antioxidant enzymes, like SOD, CAT, and GSH/GSSG, was noted, together with decreased MDA and $NO2^{-}/NO3^{-}$ levels (Lima et al. 2018).

Good penetration of ginger and its metabolites was confirmed by Martins et al. in their double-blind randomized clinical trial in 60 participants suffering from **episodic migraines** with no aura who consumed 400 mg of ginger extract or placebo in addition to an intravenous ketoprofen, to treat the attack of migraine. The patients who supplemented ginger showed better response after 1, 1.5, and 2 h ($p < 0.04$) (Martins et al. 2019).

Ginger rhizomes and ginger extracts have been traditionally used in the treatment of hypercholesterolemia and hypertension. However, scientific studies conducted in many research centers provide inconclusive results, and the overall effect of ginger administration on the lipid profile still seems to be unclear.

In a detailed meta-analysis of the published clinical trials on this topic, Pourmasoumi and colleagues concluded that the supplementation with ginger at a dose higher than 2 g per day was successful in the reduction of triacylglycerol (TAG) and low-density cholesterol (LDL) levels, with no impact on the total cholesterol or the high-density lipoprotein cholesterol (HDL-C) (Pourmasoumi et al. 2018).

37.6 Application in Food (Including Correctly Cooking Foods Rich in Phytochemicals)

Ginger (*Zingiber officinale)* is widely used in folk medicine, but also because of its characteristic taste and smell, it is popular in gastronomy. Several food products contain ginger, and it is used, e.g., in the production of beverages and cakes and as an addition to sushi. The hot and spicy taste of rhizomes can cause irritation to the throat and larynx, which reduces the consumed quantities. Several methods have been developed to reduce the sharp taste of ginger, and some processing procedures were evaluated to extend the shelf-life, increase the content of active compounds, and improve its consumption. The ginger rhizome can be heat-treated by drying, cooking, and baking as well as fermenting to deliver marinated ginger (Choiv 2019). In marinated form it is used as an additive to various types of sushi in order to clean the taste buds. The first mention of the use of ginger in foods was connected with its addition to beer and traditional gingerbread. Cooked or baked it is an excellent additive to meats, raising the health benefits of dishes and increasing antioxidant properties. Ginger in food can affect an increase in the antioxidant activity, demonstrate the protective effect on the liver, exhibit antiaging properties, and work in the prevention of stomach ulcers and cancer. Thanks to its antibacterial properties, it can prolong food durability (Chrubasik et al. 2005).

37.7 Safety: Toxicity and Side Effects

Ginger is recognized by the FDA (US Food and Drug Administration) as a food additive that is "generally recognized as safe."

However, the occurrence of adverse reactions after ingestion of ginger has been demonstrated in some studies after administration of 1 g of raw material. The most commonly described symptoms were gastrointestinal discomfort: diarrhea, heartburn, abdominal discomfort, indigestion, and nausea. The symptoms were of mild to moderate severity, and the serious effects were not recorded. There are cases describing the occurrence of asthma after inhalation of ginger by an increase in the level of IgE antibodies – in people working in the production of ginger in the form of

a spice. In ginger toxicity studies, there were no changes in blood counts, hemoglobin, liver function tests, or creatinine.

Pillai et al. (2011), investigating the use of ginger in nausea and vomiting caused by chemotherapy in children and adolescents, did not report any side effects.

In order to investigate the safety of ginger administration in pregnant women, studies were carried out on a group of women in the first trimester of pregnancy. Ginger was given to the pregnant women in the form of capsules, teas, ginger cakes, or candies; the results of the tests in comparison with the comparative group did not differ significantly. Unfavorable symptoms described were limited to abdominal discomfort, reflux, and drowsiness. However, it should be emphasized that the duration of the study was short and the number of patients small (Portnoi et al. 2003).

37.7.1 Interactions

Ginger has antiplatelet aggregation properties; in the literature, a case of interaction of ginger compounds with anticoagulants – warfarin – was described. It led to an increase in the international normalized ratio (INR) and nasal bleeding (Krüth et al. 2004). On the other hand, the studies performed by Jiang et al. (2008) did not confirm the effect of ginger combined with anticoagulants on the results of blood analysis and on the pharmacokinetics of warfarin. Ambiguous results suggest the need for further research to clearly determine the safety of ginger in patients with an increased risk of bleeding.

According to in vitro studies, extracts of ginger affected cytochrome p450 and P-glycoprotein, which can cause potential interactions with many commonly used drugs (Nabekura et al. 2005).

37.8 Patents

Ginger rhizome extracts, its single metabolites, and powdered ginger rhizome are subjects of numerous patent submissions around the world. The search engine for patents by Google showed more than 196,000 of items. Most of them describe the use of ginger extracts, as a source of gingerols and shogaols and as health-promoting food products. Some of them relate to their medical applications or the methodology describing the acquisition of high value of plant material (e.g., summer and autumn cultivation method – CN101283655A), plant extracts (JP2002047195A), or ginger preparations, e.g., teas or herbal compositions. Several submissions are related to the utility of powdered rhizome for industrial applications, e.g., as an ingredient of a stop-leak composition for automobile coolers (US4348235A), as a special purpose fertilizer (CN1463951A), as detergent (CN103351957A), or in the cosmetics industry (shampoo, CN102451128A, CN1513436A; soap, JP2004002517A; liquid soap, CN102008414A; plant hair dye, CN101559030A).

The addition of ginger to food preparations was intended to achieve the reduction of gastroesophageal reflux in infants (US6051235A); to promote liver and intestinal

health and reduce inflammation (US20010046523A1, US6387416B1); to support cardiovascular functions (US20010046523A1, CN1730094A); to reduce pain, swelling, and decreased mobility of limbs (WO2014188214A1); to protect the stem cells (EP2772245A1); to prevent from cancer (CN1337243A, WO2002098399A2), from chronic obstructive pulmonary disease, or from Parkinson's disease (CN101862312A); to induce analgesic and anti-inflammatory action in the mouth (JP2007169247A); to warm the body during cold or flu conditions (CN103385323A); and others.

Often ginger-enriched various compositions in combination with other plant species or metabolites are described. The patents apply the combinations with long pepper (CN102166335A), dark plum fruits (CN102091315A), clove (CN102145158A), or orange peel (CN102133384A) to increase the efficacy of medicaments for radiotherapy- and chemotherapy-induced emesis and with bisabolol (US20090238905A1) to intensify the anti-inflammatory potential in the gastrointestinal and respiratory tracts, to keep out cold and tonify the organism (CN102406013A), and to support the healthy swallowing (US6596313B2) and hair growth (CN1130056A), in sleep- (US6391346B1), hormonal balance- (US6242012B1), and prostate health- (US6261607B1) promoting compositions.

Ginger rhizomes or extracts are introduced to teas (CN102669332A, CN102406013A, CN1149404A; brown sugar ginger tea, CN102696805A; ginkgo and ginger tea, CN101627790A), beverages (kvass drink, CH694314A5; ginger-haw beverage, CN101066152A; ginger brown sugar beverage, CN102266108A), flour confectionery products (gingerbread RU2450525C1, RU2388229C1, CN102461621A; ginger cakes CN101617790A, CN1210679A), porridges (CN103478551A), jams (CN1234983A), noodles (CN103621895A), milk (CN1398530A), yoghurt (CN101530132A), wine (CN102559428A), honey (CN101779714A), ginger pastes (CN1246293A, CN1176069A), syrup (FR2609870A1), candies (CN103039677A), and sausages (DE3247166A1) and manufactured as fermented rhizomes (CN101273783A, KR101254740B1, CN101675781A) or beverages containing fermented ginger (KR100880127B1).

The patents list a wide range of extraction protocols of *Zingiber rhizoma* (ginger oil – CN1065782A), which in their optimized operation conditions provide enriched extracts of high pharmacological potential (e.g., anti-inflammatory, anti-fungal, antiplatelet aggregation, antiemetic properties) and longer stability (US20050031772A1, DE19859499A1, JP2012050377A). Among them supercritical carbon dioxide extraction (JP20020047195A, CN1692924A, CN1144102A), reflux extraction (CN102293997A), and steam distillation (JP2002047195A) play the most important role. Also, granulation method was elaborated for ginger (CN103609991A). Some patents (e.g., JP2012249553A) describe the constructed protocols applied to enrich the extracts in particular metabolites, e.g., increase the content of shogaols (JP2008079562A) and gingerols (JP2012050377A, CN104447259A), or decrease the presence of gingerols known from their skin-irritating properties (water-soluble ginger extract EP1281402B1), and isolate 6-gingerol from ginger extract (CN104397660A, CN1616391A) also by means of high-speed countercurrent chromatography (CN101781184A) or 6- and 8-gingerol by reflux, liquid-liquid extraction, and column chromatography (CN101085727A).

The preservation of ginger extracts properties has been also widely patented. Temperature- and humidity-controlled storage boxes were constructed to preserve the rhizomes from deterioration (CN102344019A). Moreover, its oleoresin was microencapsulated with cyclodextrin (CN101473954A) or maltodextrin and arabic gum (CN1488290A).

37.9 Perspectives

Plentiful pharmacological effects of gingerols and shogaols presented in this chapter but also denoted in numerous studies performed on in vitro models, which were not described herein, shed light on the application of ginger rhizome as a nutraceutical and a natural remedy. Ginger rhizomes, as the major source of gingerols and shogaols, were traditionally used as digestives and antimicrobials. Together with the development of the scientific instruments and assays, further precious properties of this plant were revealed. Its administration in human seems to be an interesting and precious alternative to synthetic drugs, which often bear serious side effects. Low toxicity of single metabolites of ginger extracts and often appearing synergistic actions of its metabolites with synthetic drugs encourages the use of ginger metabolites in various therapeutic strategies. Thanks to a wide spectrum of pharmacological studies on ginger, it may be concluded that the spice has a value of functional foods.

Among health benefits of ginger, its value in the cancer treatment should be underlined. The oleoresin constituents are also effective in the antiobesity, antidiabetic, antisclerotic, and antiamnestic treatment and also as antioxidants, chemopreventives, and anti-inflammatory agents.

Therefore, it is of the highest importance to collect funds on the structure-activity studies, reveal the potential of minor gingerols and shogaols present in the extracts, and collect funds and volunteers on the clinical trials on ginger extracts and metabolites.

This still underestimated spice will certainly draw attention of researchers in the nearest future, as further trials are necessary to confirm its therapeutic benefits already revealed in in vitro tests.

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Organosulfur Compounds in Food

38

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Contents

Abstract

Sulfur is one of the essential macronutrients and plays critical role in overall health maintenance. Plant-derived organosulfur compounds (OSCs) are the important source of dietary sulfur. Garlic is one of the OSCs-enriched vegetables with long history of cultivation and has been widely applied in nutritional, healthcare, and medicinal applications. The potential functions of garlic and garlic derivatives in health beneficial and disease prevention have been well accepted by the people worldwide. In this chapter, taking garlic as a case, the research progress (up-to-date 2019) on the investigations of phytochemistry,

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bioavailability and metabolism, pharmacology, and safety for garlic OSCs were systemically reviewed and discussed. Moreover, the information on marketed products and granted patent (2009–2018) for garlic derivative products were also summarized.

Keywords

Organosulfur compounds · Garlic · Phytochemistry · Pharmacology · Safety

38.1 Introduction

Sulfur is one of the essential macronutrients (the seventh most abundant) to human, and widely distributed in nearly all human proteins, in building blocks of the body's tissues and organs (Jalilehvand 2006). Sulfur is also an important part of some biological coenzymes (e.g., *S*-adenosyl methionine; protease, ATP carboxylase; and acetyl coenzyme A), several amino acids (e.g., methionine, cysteine, homocysteine, and taurine), peptides (e.g., glutathione), and vitamins (e.g., vitamin B, vitamin H, and carotene) and plays numerous critical roles in metabolism and overall health maintenance (Dong et al. 2018; Ingenbleek and Kimura 2013). Although poorly studied, deficiency of sufficient amount of sulfur has been considered to be one of the potential factors responsible for increasing risk of obesity, chronic fatigue, cardiovascular disorders, dermatological problems, allergies, and even neurodegenerative Alzheimer's disease (Seneff 2010). Therefore, daily uptake of certain amount of sulfur element is considered to be necessary and benefit to maintain good health.

In nature, sulfur presents as various forms (inorganic and/or organic) in different foods. High-protein foods, such as meats, poultry, fish, legumes, cheese, eggs, and nuts, are the primary source of sulfur-containing amino acids (SAAs). Moreover, because of the fact that biosynthesis of organosulfur compounds (OSCs) takes place only in plants and bacteria, plant-sourced OSC is another important source of sulfur both to human and animals (Prasad 2014). The OSC-enriched plant foods include allium vegetables (e.g., garlic, onion, chive, scallion, shallots, and leek) and cruciferous vegetables (e.g., turnip, broccoli, cabbage, and cauliflower), other vegetables (e.g., spinach, tomatoes, carrot, mustard, and potatoes), fruits (e.g., avocados, bananas, watermelon, and pineapple), as well as cereals (e.g., brown rice and wheat flour) (Petropoulos et al. 2017).

Garlic (*Allium sativum* L.) is one of the worldwide-distributed and historically cultivated allium vegetables and has been widely applied in the fields of nutrition, healthcare, and medicines (Santhosha et al. 2013). In chemistry, garlic contains an abundance of OSCs; carbohydrates, proteins, and fiber; trace elements; and compounds of phenolic and steroidal origin (Santhosha et al. 2013). Among them, OSCs have been identified to be closely associated to various bioactivities of garlic, including antimicrobial (Goncagul and Ayaz 2010), anticancer (Ariga and Seki 2006), antioxidant (Capasso 2013), antidiabetic (Hosseini and Hosseinzadeh 2015), hepatoprotective (Guan et al. 2018), immune boosting (Arreola et al. 2015),

as well as potentials in preventing cardiovascular diseases (Zhu et al. 2018). In this chapter, we mostly deal with OSCs that are found in garlic (*Allium sativum* L.) for their phytochemistry, bioavailability and metabolism, pharmacology, safety, and applications.

38.2 Bioactive Constituents

Alliin (*S*-alk(en)yl cysteine sulfoxide) is an important natural ingredient which separately exists with alliinase in fresh garlic. When the garlic was chopped or crushed, alliin will be quickly converted to allicin (γ-glutamyl-*S*-alkenyl-L-cysteine) by alliinase. Thereafter, allicin is converted readily to some more stable alk(en)yl sulfides, vinyldithiins, ajoenes, and some others (Fig. 1) (Guan et al. 2018; Amagase 2006).

Currently, various organosulfur compounds (OSCs) have been isolated and identified from fresh and/or processed garlic. Based on their physicochemical properties, the garlic OSCs could be divided into lipid-soluble (essential oil) and water-soluble compounds. The mostly investigated lipid-soluble compounds include alliin, allicin, such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and ajoene, while the most investigated water-soluble compounds include *S*-allyl-L-cysteine (SAC), *S*-allylmercaptocysteine (SAMC), and allyl mercaptan (AM). The chemical structures of some representative OSCs from garlic were illustrated in Table 1.

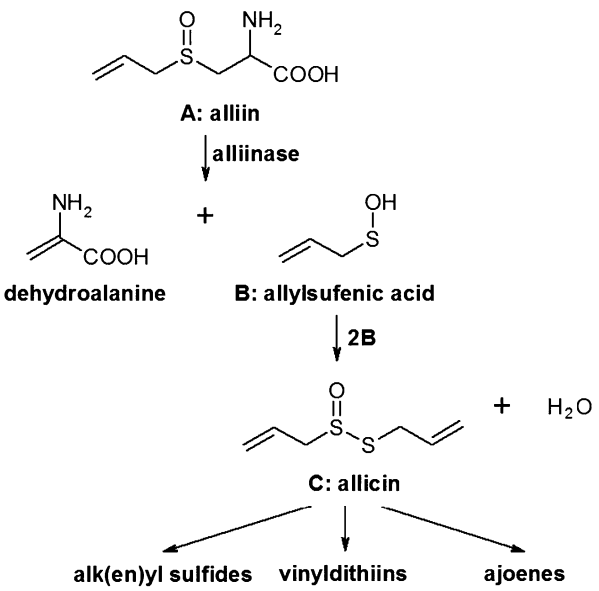
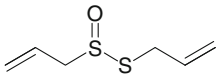
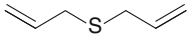
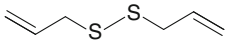
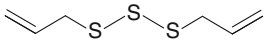
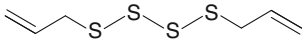
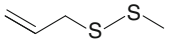
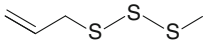
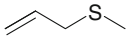
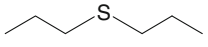
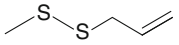
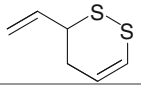
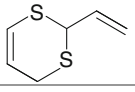
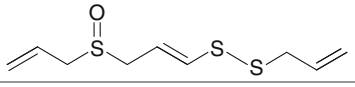
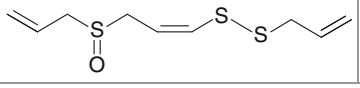
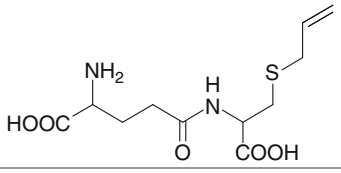
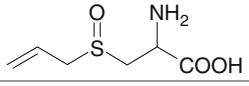


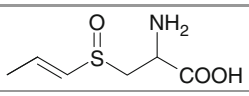
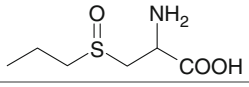
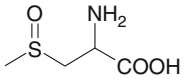
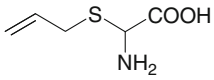
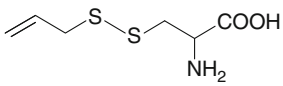
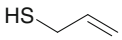
Fig. 1 The enzymatic reaction of alliin (*S*-allyl cysteine sulfoxide) and further reaction of allicin (γ-glutamyl-*S*-alkenyl-L-cysteine)

Table 1 The chemical structures of representative organosulfur compounds (OSCs) from garlic

No.	Compound	Chemical structure	Molecular formula	References
Lipid-soluble				
1	Diallyl thiosulfinate (allicin)		$C_6H_{10}OS_2$	Guan et al. (2018)
2	Diallyl sulfide (DAS)		$C_6H_{10}S$	Guan et al. (2018)
3	Diallyl disulfide (DADS)		$C_6H_{10}S_2$	Guan et al. (2018)
4	Diallyl trisulfide (DATS)		$C_6H_{10}S_3$	Guan et al. (2018)
5	Diallyl tetrasulfide (DATTS)		$C_6H_{10}S_4$	Yagdi et al. (2016)
6	Allyl methyl disulfide (AMD)		$C_4H_8S_2$	Guan et al. (2018)
7	Allyl methyl trisulfide (AMT)		$C_4H_8S_3$	Guan et al. (2018)
8	Allyl methyl sulfide (AMS)		C_4H_8S	Yagdi et al. (2016)
9	Propyl disulfide		$C_6H_{14}S_2$	Gîtin et al. (2014)
10	Allyl methyl sulfide		C_4H_8S	Gîtin et al. (2014)
11	2-vinyl-4H-1,3-dithiin		$C_6H_8S_2$	Guan et al. (2018)
12	3-vinyl-4H-1,2-dithiin		$C_6H_8S_2$	Guan et al. (2018)
13	(*E*)-ajoene		$C_9H_{14}OS_3$	Foroutan-Rad et al. (2017)
14	(*Z*)-ajoene		$C_9H_{14}OS_3$	Foroutan-Rad et al. (2017)
Water-soluble				
15	γ-Glutamyl-*S*-allyl-L-cysteine (GSAC)		$C_{11}H_{18}N_2O_5S$	Guan et al. (2018)
16	*S*-alk(en)yl-L-cysteine sulfoxides (alliin)		$C_6H_{11}NO_3S$	Foroutan-Rad et al. (2017)

(continued)

Table 1 (continued)

No.	Compound	Chemical structure	Molecular formula	References
17	*S*-propenyl-L-cysteine sulfoxide (isoalliin)		$C_6H_{11}NO_3S$	Ichikawa et al. (2006)
18	*S*-propyl-L-cysteine sulfoxide		$C_6H_{13}NO_3S$	Mazelis and Crews (1968)
19	*S*-methyl-L-cysteine sulfoxide		$C_4H_9NO_3S$	Mazelis and Crews (1968)
20	*S*-allyl-L-cysteine (SAC)		$C_5H_9NO_2S$	Foroutan-Rad et al. (2017)
21	*S*-allylmercaptocysteine (SAMC)		$C_6H_{11}NO_2S_2$	Foroutan-Rad et al. (2017)
22	Allyl mercaptan		C_3H_6S	Lawson and Hunsaker (2018)

38.3 Bioavailability and Metabolism

The biopharmaceutical properties of garlic OSCs are limitedly investigated and reported because most of OSCs could not be directly determined in biological samples, which are due to their metabolic instability after administration.

In one early study in human, breath acetone and breath allyl methyl sulfide (AMS) were used as makers to evaluate the oral bioavailability of allicin. The AMS was further identified to be the main active metabolite of allicin and allicin-derived compounds in vivo (Lawson and Wang 2005; Lawson and Gardner 2005). In addition, in a mice model, allixin was detected to be quickly metabolized within 2 h and with an oral bioavailability of 31%. The main metabolites of allixin were identified to present a hydroxylated pentyl group in the structure (Kodera et al. 2002a).

Recently, the pharmacokinetic profiles of SAC, one of the most abundant components in garlic OSCs, have been widely investigated and reported in animals (mice, rat, and dog) (Park et al. 2017; Amano et al. 2015, 2016; Lee et al. 2015). SAC was detected to be fast and completely absorbed from the gastrointestinal tract (bioavailability >90%) (Amano et al. 2015) and extensively reabsorbed from the kidneys. The main metabolites of SAC were determined to be *N*-acetyl-(*S*)-allyl-L-cysteine (80%) and *N*-acetyl-(*S*)-allyl-L-cysteine sulfoxide (11%), which were excreted with SAC (2.9%) in urine (Amano et al. 2016). In addition, another study compared the bioavailability and bioequivalence of allicin after oral administration

of different garlic supplements and garlic foods (Lawson and Hunsaker 2018). The obtained results provided a new guideline for developing of the quality standard for manufacturers of garlic products.

38.4 Bioactivities

The OSCs have been investigated to present various pharmacological activities. The main biological properties of garlic OSCs include antimicrobial, anti-inflammatory, antioxidant and immunomodulatory, anticancer activities, as well as cardiovascular protection.

38.4.1 Antimicrobial Activity

The antimicrobial activity of garlic has long been recognized and investigated (Goncagul and Ayaz 2010). Garlic juice was considered to be "natural antibiotic" and ever used as disinfectant to prevent wound infections during the World Wars. The antimicrobial activities of garlic are closely related to its high contents of OSCs (Kyung 2012). Some garlic-derived OSCs have been reported to present broad-spectrum activities against bacterial, viral, mycotic, and parasitic infections (Goncagul and Ayaz 2010).

In some researches, the volatile oil and/or alcohol extracts have been investigated to present much higher antimicrobial activities than those of the water extracts for garlic. Further studies indicated that garlic OSCs played important roles in inhibiting bacteria (gram-positive and gram-negative), fungus, parasites, and viruses (Kshirsagar et al. 2018; Foroutan-Rad et al. 2017; Borlinghaus et al. 2014; Ankri and Mirelman 1999; Naganawa et al. 1996; Weber et al. 1992). The relationship of the main garlic OSCs and their antimicrobial properties are summarized in Table 2.

Recently, allicin was reported to be more potent against pathogens of certain drug-resistant bacteria such as methicillin-resistant *S. aureus* than some traditional antibiotics (Petropoulos et al. 2017). The potential mechanisms might be related to the combined approaches by decreasing glutathione levels, unfolding stress, and inactivation of crucial metabolic enzymes via S-allylmercapto modification of cysteines (Müller et al. 2016).

Except for the direct actions, garlic OSCs were also observed to enhance the antifungal activity of some other antimicrobials. It was reported that allicin could synergistically enhance the fungicidal activity of amphotericin B against *S. cerevisiae* through effecting on the intracellular ergosterol trafficking (Ogita et al. 2009). In addition, allyl sulfide significantly increased the sensitivity of microbial to multiple antimicrobial agents through modulating multidrug efflux pump EmrD-3 as well as in synergy with multiple antimicrobial agents (Bruns et al. 2017).

Table 2 Antimicrobial activities of main OSCs from garlic

Antimicrobial activity	Garlic-derived compound	References
Antibacterial		
Gram-positive bacteria		
Staphylococcus aureus	Allicin, allistain I, allistain II, ajoene	Goncagul and Ayaz (2010), Ankri and Mirelman (1999), Naganawa et al. (1996)
Streptococcus spp.	Allicin, ajoene	Borlinghaus et al. (2014), Ankri and Mirelman (1999), Naganawa et al. (1996)
Bacillus spp.	Allicin, ajoene, DADS	Borlinghaus et al. (2014), Naganawa et al. (1996)
Mycobacterium spp.	Ajoene	Naganawa et al. (1996)
Lactobacillus spp.	Allicin, ajoene	Naganawa et al. (1996)
Gram-negative bacteria		
Vibrio cholerae	Allicin	Borlinghaus et al. (2014)
Escherichia coli (E. coli)	Allicin, allistain I, allistain II, ajoene	Goncagul and Ayaz (2010), Borlinghaus et al. (2014), Ankri and Mirelman (1999), Naganawa et al. (1996)
Salmonella typhimurium	Allicin	Borlinghaus et al. (2014)
Pseudomonas aeruginosa	Allicin, ajoene	Ankri and Mirelman (1999), Naganawa et al. (1996)
Proteus mirabilis	Allicin	Ankri and Mirelman (1999)
Acinetobacter baumannii	Allicin	Ankri and Mirelman (1999)
Helicobacter pylori	Allicin	Goncagul and Ayaz (2010)
Agrobacterium tumefaciens	Allicin	Borlinghaus et al. (2014)
Pseudomonas syringae	Allicin	Borlinghaus et al. (2014)
Klebsiella pneumoniae	Allicin, ajoene	Ankri and Mirelman (1999), Naganawa et al. (1996)
Antifungal		
Candida albicans	Allicin, ajoene, DADS	Goncagul and Ayaz (2010), Ankri and Mirelman (1999), Naganawa et al. (1996)
Histoplasma capsulatum	Allicin	Goncagul and Ayaz (2010)
Aspergillus	Allicin	Goncagul and Ayaz (2010)
Trichophyton spp.	Allicin, ajoene	Goncagul and Ayaz (2010)
Cryptococci spp.	Allicin	Goncagul and Ayaz (2010)
Penicillium	Allicin	Goncagul and Ayaz (2010)
Torulopsis glabrata	Allicin	Ankri and Mirelman (1999)
Candida spp.	Allicin, ajoene	Goncagul and Ayaz (2010)
Saccharomyces cerevisiae	Ajoene	Naganawa et al. (1996)

(continued)

Table 2 (continued)

Antimicrobial activity	Garlic-derived compound	References
Hanseniaspora valbyensis	Ajoene	Naganawa et al. (1996)
Pichia anomala	Ajoene	Naganawa et al. (1996)
Schizosaccharomyces pombe	Ajoene	Naganawa et al. (1996)
Saccharomyces cerevisiae	Ajoene	Naganawa et al. (1996)
Antiviral		
Human cytomegalovirus	Allicin	Ankri and Mirelman (1999)
Influenza B	Allicin	Ankri and Mirelman (1999)
Herpes simplex virus type 1	Ajoene, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate	Ankri and Mirelman (1999), Weber et al. (1992)
Herpes simplex virus type 2	Ajoene, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate	Ankri and Mirelman (1999), Weber et al. (1992)
Vaccinia virus	Ajoene, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate	Ankri and Mirelman (1999), Weber et al. (1992)
Parainfluenza virus type 3	Ajoene, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate	Ankri and Mirelman (1999), Weber et al. (1992)
Vesicular stomatitis virus	Ajoene, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate	Ankri and Mirelman (1999), Weber et al. (1992)
Human rhinovirus type 2	Ajoene, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate	Ankri and Mirelman (1999), Weber et al. (1992)
Antiparasitic		
Entamoeba histolytica	Allicin	Ankri and Mirelman (1999)
Giardia lamblia	Allicin	Ankri and Mirelman (1999)
Leishmania major	Allicin	Ankri and Mirelman (1999)
Leptomonas colosoma	Allicin	Ankri and Mirelman (1999)
Crithidia fasciculata	Allicin	Ankri and Mirelman (1999)

38.4.2 Anti-inflammatory, Antioxidant, and Immunomodulatory Activities

Oxidative stress-induced inflammatory reactions are always occurring in many chronic autoimmune diseases (Lingappan 2018; Sies et al. 2017; Lugrin et al. 2014). Effective suppression of inflammatory cytokines and scavenge of reactive oxygen species (ROS) are helpful for improving the clinical symptoms and progression of such diseases. In many studies, the anti-inflammatory, antioxidant, and immunomodulatory activities of garlic OSCs have been investigated and reported (Colín-González et al. 2015). The most investigated compounds include DAS

(Suman and Shukla 2016), DADS (Liu et al. 2018), DATS (Zhang et al. 2016), allicin (Zhang et al. 2017), alliin (Shi et al. 2017), *S*-allyl cysteine (Colín-González et al. 2015), as well as some others.

In vitro, OSCs were observed to effectively suppress the release of nitric oxide (NO) (Liu et al. 2014), secretion of various proinflammatory cytokines (e.g., TNF-α, IL-6, IL-8, IL-1β, etc.) (Liang et al. 2019), and generation of ROS (Zhang et al. 2016) in various inflammatory cell models. In vivo, OSCs exhibited potentials in improving clinical symptoms and diseases progression for chronic inflammatory diseases (including arthritis, colitis, diabetic nephropathy, liver injures, neuroinflammations, cardiovascular inflammations, as well as some infectious inflammations) both in animals and humans. Biologically, the underlying pharmacological mechanisms of such activities were demonstrated to be related to their modulations on endothelial nitric oxide synthase (Liu et al. 2014), nuclear factor kappa-B (NF-κB) pathway (Liang et al. 2019), mitogen-activated protein kinases (MAPKs) pathway (Shi et al. 2017), and oxidative stress (Nrf2) pathway (Zhang et al. 2017). All these studies suggested great potentials of OSCs for their further development and application as on autoimmune inflammatory diseases.

38.4.3 Anticancer Activity

In the past decades, the anticancer activity of garlic extracts and OSCs has been widely investigated by many researchers. A numerous cell and animal studies reported that garlic and garlic-derived OSCs presented anticancer activity against various cancers (e.g., breast, gastric, colorectal, liver, glioma, lung, and some other cancers) (Nohara et al. 2017; Puccinelli and Stan 2017; Nicastro et al. 2015). In clinical trials, although several inverse reports, garlic intake was still considered to be potentially associated to the reduction on the risk of some cancers (Wu et al. 2019; Guercio et al. 2014; Kim and Kwon 2009). Furthermore, OSCs were identified to be the most important bioactive compounds to be responsible for the anticancer activity for garlic (Lugrin et al. 2014; Schäfer and Kaschula 2014; Cao et al. 2014; Tsubura et al. 2011).

38.4.3.1 Breast Cancer

To be a most common cancer among females worldwide, breast cancer is estimated to impact more than 2.1 million women each year. In 2018, reported by WHO, about 627,000 women died from breast cancer (approximately 15% of all cancer deaths among women). In some studies, garlic OSCs were demonstrated to affect the development and progression of breast cancer both in vitro and in vivo (Tsubura et al. 2011). Diallyl disulfide (DADS), one of the most important OCSs in garlic, was reported to regulate multiple cancer hallmark pathways involved in cell cycle, apoptosis, invasion, and metastasis in ER-positive and/or ER-negative breast cancers (Xiong et al. 2018). In addition, some other OSCs such as DATS (Puccinelli and Stan 2017), alliin (Izdebska et al. 2016), and SAMC (Zhang et al. 2014) were also reported to present multiple antitumor activities by inducing cell apoptosis and inhibiting cancer metastasis.

38.4.3.2 Digestive (Gastric, Colorectal, and Liver) Cancers

The preventive and therapeutic activities of garlic on digestive cancers have been widely investigated and reported. Garlic OSCs presented satisfied effects on management of various gastric, colorectal, and liver cancers through multiple biological mechanisms. Antioxidative, anti-inflammatory, antiproliferative, and apoptotic effects were demonstrated to be involved in the pharmacological and biological properties of garlic for digestive cancers (Nohara et al. 2017; Nicastro et al. 2015; Fukushima et al. 1997). In recent years, however, the relationship between garlic consumption and reduced risk of gastric and/or colorectal cancers was controversially reported by many studies (Wu et al. 2019; Guercio et al. 2014; Kim and Kwon 2009). Although long-term intake of OSC-enriched garlic products is considered to be beneficial for managements of digestive tract cancers, further epidemiological evidence needs to be conducted to better clarify the relationship between garlic intake and risk of digestive tract cancer occurrence.

38.4.3.3 Brain Cancers

The anticancer effects of garlic OSCs on brain cancers were investigated using various brain cancer models. Garlic OSCs (DAS, DADS, DATS, allicin, and *Z*-ajoene) were investigated to exhibit good effects to promote apoptosis of human glioblastoma cells (T98G, U87MG, SH-SY5Y, and U251) in vitro (Jurkowska et al. 2017) and prevent tumor progression in U87MG-xenografted mice in vivo (Wallace et al. 2013) through antiproliferation, apoptosis, anti-inflammation, and antioxidation. Moreover, for the high liposolubility in physiological fluid, some OSCs might easily cross through the blood-brain barrier and enter into the brain for treatment of brain cancers.

38.4.3.4 Lung Cancer

The anticancer effects of garlic OSCs were also investigated in lung cancer, using various lung cancer models (Nohara et al. 2017; Nicastro et al. 2015). The isolated sulfur-containing compounds of DADS, DATS, SAMC, and ajoene from garlic were found to be effective on lung cancers both in vitro and in vivo. Moreover, because of the volatile properties, OSCs might be pharmaceutically inhaled to be topically delivered to lung tissue thus presenting direct actions in treating lung cancers.

38.4.3.5 Other Cancers

Moreover, the garlic OSCs have been reported to present anticancer activities against some other cancers. DADS and DATS were observed to inhibit differentiation and induce apoptotic death of leukemia both in vitro and in vivo (Sun et al. 2019; Hung et al. 2015). Allicin, SAC, and SAMC were determined to suppress cell proliferation and differential responses of ovarian cancer (Wu et al. 2016).

38.4.4 Cardiovascular Protection

Cardiovascular protection is one of the most important functions for garlic-derived products. The protective effects of garlic on cardiovascular health have been long time investigated and reported (Zhu et al. 2018; Varshney and Budoff 2016). Numerous studies indicated that OSCs play pivotal role regarding the beneficial effects in reducing the risk of cardiovascular diseases (CVD) (Schwingshackl et al. 2016; Seki and Hosono 2015). Pharmacologically, OSCs could effectively prevent or improve CVD through multiple approaches, mainly including antihyperlipidemia (Ried 2016), antihypertension (Ried 2016), and antiplatelet aggregation and antithrombosis (Ariga and Seki 2006).

38.4.4.1 Antihyperlipidemia

Hyperlipidemia is a latent dangerous pathological factor to the development of atherosclerosis. Elevated serum total cholesterol (TC) and/or triglyceride (TG) and reduced high-density lipoprotein cholesterol (HDL-C) are the important clinical indicators for hyperlipidemia (Jain et al. 2007).

The lipid-lowering effects of garlic have long been focused by experimental and clinical attentions. Various in vitro (Chang et al. 2015; Lii et al. 2012) and in vivo (Yang et al. 2018; Annamalai et al. 2017) investigations indicated that garlic oil (high content of OSCs) could effectively improve hyperlipidemia through regulating serum TC and TG as well as HDL-C. However, in clinical trials, although some data are inconsistent (which might be duo to the lower doses of garlic used), the antihyperlipidemic effects of garlic were observed according to the results obtained from some meta-analyses. Compared to garlic, garlic powder and aged garlic extract were found to be more effective in reducing serum TC levels, while garlic oil was more effective in lowering serum TG levels (Zeng et al. 2012).

38.4.4.2 Antihypertension

Hypertension (HTN), high blood pressure, is a major modifiable risk factor for CVD. Unmanaged HTN might result to heart attack, stroke, chronic heart failure, renal failure, and some other problems (Drozdz and Kawecka-Jaszcz 2014). Since the bioactive properties of garlic on anti-HTN have been investigated to be analogous to those of certain standard drugs, garlic derivative (powder, extracts, oil, and/or OSCs) becomes one of the most studied functional food and is being widely used as dietary supplements by people worldwide for ameliorating HTN (Ried and Fakler 2014).

Based on numerous studies, multiple mechanisms underlying have been identified to be involved in the antihypertensive activities of garlic bioactive ingredients (Ushijima et al. 2018). The main mechanisms include (1) improvement of redox balance and cellular homeostasis; (2) suppression of NF-κB expression in endothelial cells; (3) lowering cytosolic and mitochondrial ROS concentration; (4) increasing H_2S production by modulating cystathionine c-lyase (CSE) activity; (5) stimulation of NO production in blood vessels to relax and improve elasticity of

vascular smooth muscle; (6) inactivation of renin-angiotensin-aldosterone (RAA) system by inhibiting angiotensin-converting enzyme (ACE) activity; and (7) prevention of excessive growth of vascular smooth muscle cells (VSMCs). Moreover, sulfur-containing compounds such as allicin, DADS, DATS, and S-allyl cysteine have been attributed to the antihypertensive activities of garlic (Ried and Fakler 2014; Shouk et al. 2014).

38.4.4.3 Antithrombosis and Antiplatelet Aggregation

Garlic has been reported to inhibit platelet aggregation through various mechanisms such as inhibition of cyclooxygenase activity thus leading to thromboxane A2 formation; suppression of calcium mobilizing into the platelets; and increase messenger (cAMP and cGMP) levels within platelets (Bradley et al. 2016; Qidwai and Ashfaq 2013). Moreover, garlic extract could also inhibit the aggregation of platelets by improving dissolution of clots and thrombi via fibrinolysis (Qidwai and Ashfaq 2013).

For example, simultaneously facilitated extracellular ATP and extracellular/intracellular TXB2, as well as suppressed collagen-induced ERK, p38, and JNK phosphorylation, were observed in aged garlic extract (AGE)-treated platelets, which suggested that AGE could suppress platelet aggregation by changing the functional property of platelets (Morihara and Hino 2017). Among the compounds of garlic, allicin and its self-condensation product (ajoene) were considered to be the primary substances responsible for the functions of antithrombosis and antiplatelet aggregation (Chan et al. 2013; Teranishi et al. 2003). All the evidence suggested the effects of OSCs on thrombosis and platelet aggregation for their potentials in preventing cardiovascular diseases.

38.4.5 Others

Except for the abovementioned biological activities, other pharmacological activities of garlic and garlic OSC products are also investigated and reported. The neuroprotection of aged garlic extract and SAC on improving inflammation-induced cognitive dysfunction was identified by in vivo examinations (Nillert et al. 2017; Zarezadeh et al. 2017). In addition, garlic extracts and OSCs (alliin, allicin, DADS, DATS, DAS, SAC, ajoene, and allyl mercaptan) presented great potentials to be developed as promising agents for the management of diabetics and its complications (Padiya and Banerjee 2013).

38.5 Benefits (Human Studies)

Due to the multiple bioactivities, the benefits of garlic and garlic OSCs to human health have been long-term investigated and observed. Among these studies, the benefits of garlic derivative products for preventive and therapeutic effects on cardiovascular diseases (CVD) and cancers are mostly concerned.

The association between uptake of garlic and reduction of CVD risks has been widely investigated and assessed by numerous clinical trials in humans (Zhu

et al. 2018; Varshney and Budoff 2016). Most studies demonstrated the main responsibility and importance of garlic OSCs in cardiovascular protection (Schwingshackl et al. 2016; Seki and Hosono 2015). The pharmacological effects of OSCs for treatment or prevention of CVD have been established to be closely related to its multiple bioactivities including antioxidation, anti-inflammation, immunomodulation, antihyperlipidemia, antihypertension, antiplatelet aggregation, and antithrombosis (Ried 2016; Zeng et al. 2012). In recent years, some emerging evidence indicated the cardioprotective and cytoprotective properties for hydrogen sulfide (H_2S) (Bradley et al. 2016). Since some garlic OSCs could be readily degraded into organic diallyl polysulfides (the potent donors for H_2S) in the presence of thiols, H_2S-dependent hypothesis might provide a new insight for garlic OSCs in preventing myocardial injury and dysfunction (Bradley et al. 2016).

The relationship between garlic consumption and reduced cancer risk is a subject of considerable controversy. Some studies reported the positive relevance between them (Wu et al. 2019; Guercio et al. 2014; Kim and Kwon 2009), while some results suggested the irrelevance. Whatever, the chemopreventive properties (inhibition of the processes of carcinogenesis through anti-inflammation, anti-transformation, and antiproliferation; effects on the final phase of carcinogenesis through inhibiting angiogenesis and metastasis) of garlic have attracted considerable attention in some adjuvant therapies for cancer control (Schäfer and Kaschula 2014). Currently, various garlic and garlic OSC products have been developed as adjuvant agents with immunomodulatory effects for decreasing the side effects and/or enhancing the therapeutic effects of some anticancer agents.

38.6 Application in Food

Garlic is an OSC-enriched dietary vegetable which is widely distributed worldwide. Various flavor foods of garlic could be found in the market of different countries. The most popular garlic foods include spicy pickled garlic, savory garlic, garlic spread, garlic rub, garlic powder, roasted garlic pieces, fried garlic clove, and fried minced garlic. The compositions and contents of OSCs in each product depend on the techniques during individual manufacturing process.

Garlic is widely used as food additive because of its aromatic smell and taste derived from OSCs. Garlic salt, spicy garlic mustard, garlic-infused white balsamic vinegar, various garlic pickles, and garlic-pickled mixed vegetables are the most representative garlic-containing foods sold in supermarkets. Moreover, garlic essential oil is also a commonly used flavoring in cooking.

38.7 Safety: Toxicity and Side Effects

To be a dietary vegetable, garlic is considered to be very safe. Currently, the available toxicological data about garlic and garlic-derived compounds are rather limited.

Reported by Kodera et al. (2002b), SAC presented very minor acute and subacute toxicities in mice and rats (LD_{50} value >54.7 mM/kg, po; and > 20 mM/kg, ip). In another toxicological study, propyl propane thiosulfinate was determined to be not mutagenic or genotoxic in vitro (Mellado-García et al. 2017). In a subacute study in rats, oral administration of high OSCs contained allium extract (0, 25, 100, 400 mg/kg/d) for 90 days, and no treatment-related clinical signs, mortality, or effects (biochemical, hematological, and histopathology parameters) were observed for all animals (Mellado-García et al. 2016).

In a clinical study, gastric administration of garlic powder was observed to induce immediate epigastric symptoms of pressure, stinging, and warmth, as well as fundic relaxation, but does not influence mechanosensitivity or compliance. This might be due to the presence of allicin in the powder (Führer et al. 2019).

38.8 Marketed Products

The OSC-enriched garlic derivatives (garlic powder, garlic oil, garlic water extract, and garlic alcohol extracts) are important raw or supplementary materials for nutritional, healthcare, and medicinal applications. Various garlic organosulfur-contained products have been commercialized in marketing. Some representative products as well as their chemical compositions, functions, and manufactures are listed in Table 3.

38.9 Patents

In the past years, numerous patents about the preparation methods and pharmacological and functional applications for organosulfur compounds have been applied and granted worldwide. Most of them are related to garlic and/or garlic derivatives. Up-to-date, over 20,000 patents for garlic and garlic derivative products have been granted in different countries and regions. Most of which are mainly distributed in China, the USA, Japan, and Europe (Table 4). The trends of the approved patents in different applications (2009.01.01–2018.12.31) in China, the USA, Japan, and Europe are illustrated in Fig. 2.

38.10 Perspectives

Plant-derived organosulfur compound (OSC) is one of the main sources of dietary sulfur and has been investigated to play important role in maintenance of human health. Garlic and its derivates are highly concerned and widely applied as the raw or supplementary materials in various nutritional, functional, and medicinal products because of their high contents of OSCs. The most investigated biological functions for garlic OSCs are mainly focused on antimicrobial, anti-inflammatory, antioxidant and immunomodulatory, and anticancer activities as well as cardiovascular protection. Although the current experimental evidence on the pharmacological activities

Table 3 Representative OSC-enriched garlic products in market

Products	Active ingredient	Functions	Manufacture
Doppelherz Garlic Capsules with Hawthorn	60 mg garlic essence,54 mg hawthorn extract	Clear blood, prevent cancer, improve immunity, prevent Alzheimer's disease	Doppelherz® (GERMAN)
Nutrilite® Garlic and Licorice	Three tablets per day provide 1000 mg of garlic bulb powder and 21 mg of licorice extract	Provides phytonutrient plant compounds for additional nutritional benefits; garlic and licorice are traditionally used to promote well-being	Nutrilite® (AMERICAN)
Nature Made® Odor Controlled Garlic Tablets	Each tablet contains 500 mg of concentrated garlic bulb	Help support normal blood cholesterol levels already within the normal range. The gentle release of garlic in the body results in virtually no after odor on the breath or on the skin	Nature Made® (AMERICAN)
The soft capsule of garlic essential oil of Tomson Beijian®	Takes garlic essential oil as the main raw material and contains 100 g of allicin, 1 g of which each contains 5 mg of allicin	Enhancing immune	Tomson Beijian® (CHINESE)
Mega Garlic Plus	Garlic powder extract 600 mg	Supports healthy circulation and heart health	Herbalife® (AMERICAN)
Odorless Super Garlic	Deodorized garlic bulb Powder (*Allium sativum*) 1000 mg, aged garlic bulb extract (*Allium sativum*), 100 mg	Supports cardiovascular health	GNC Herbal Plus® (AMERICAN)
Puritan's Pride Odorless Garlic & Parsley	500 mg/100 mg	Used for heart and cardiovascular function, respiratory health, contains breath-freshening parsley and the plant nutrient chlorophyll	Puritan's Pride
Pure Garlic	1,500 mg per softgel	Boost the immune system, supports cardiovascular health, promotes healthy cholesterol levels	NaturaLife Labs (AMERICAN)
Sundown Naturals Odorless Garlic	1000 mg, 250 odorless softgels	Facilitating better circulation and promotes good functioning of heart	Sundown Naturals (AMERICAN)
Nature's Bounty Garlic Extract	1000 mg, 100 rapid release softgels	Supports circulatory function	Nature's Bounty (AMERICAN)
Himalaya Garlic	Organic garlic powder (aerial parts) 750 mg, organic garlic extract (bulb) 650 mg	Garlic offers a broad range of body supportive mechanisms, supports normal cardiovascular	Himalaya (AMERICAN)

(continued)

Table 3 (continued)

Products	Active ingredient	Functions	Manufacture
		function, supports normal serum cholesterol levels	
Garlic (organically grown)	Proprietary blend 580 mg (garlic +parsley)	Supports healthy circulatory, liver, lung, and immune system function, provides antioxidant activity	Standard Process (AMERICAN)
The Garlic Lover	Organic canola oil, garlic essential oil	One spray while cooking, perhaps a second before you serve, will unleash the full flavor of herbs that will take your meals simply beyond	Eco-friendly (AMERICAN)
Odorless Garlic	1200 mg (250 softgels)	Support cardiovascular health, healthy circulation support and heart health support, also helps support healthy cholesterol levels within a normal range	Horbaach (AMERICAN)
Odorless Garlic	1000 mg Odorless garlic, 200 mg parsley seed oil, 56mcg chlorophyll	Support heart and cardiovascular health and may nutritionally help support cholesterol levels	BRI Nutrition (AMERICAN)
Garlic Complex	Garlic powder 1 g, spearmint oil 3 mg, rosemary extract 50mgs	Help retain normal cholesterol levels	Shaklee (AMERICAN)

Table 4 Distribution of the granted patents (2009.01.01-2018.12.31) for garlic and garlic-derived compounds in China, the USA, Japan, and Europe

	China	USA	Japan	Europe
Preparation methods	15,649	113	49	39
Medicinal applications	5227	379	313	105
Healthcare products	3647	98	222	12

on garlic OSCs is still limited, the potential functions of garlic derivatives on healthcare and disease prevention are well accepted for their long history in cultivation and traditional applications.

In chemistry, *S*-alk(en)yl cysteine sulfoxides (SACSs) are the precursor OCSs mainly presented in raw intact garlic and could be quickly degraded by enzyme alliinase into various stable small OCSs in chopped or crushed garlic. The compositions and contents of individual OCS in different garlic products were significantly different depending on the process of manufacturing. Therefore, development of the standardized processing methods for garlic derivative products and establishment of the internationally recognized standard for their quality control are necessary and urgent.

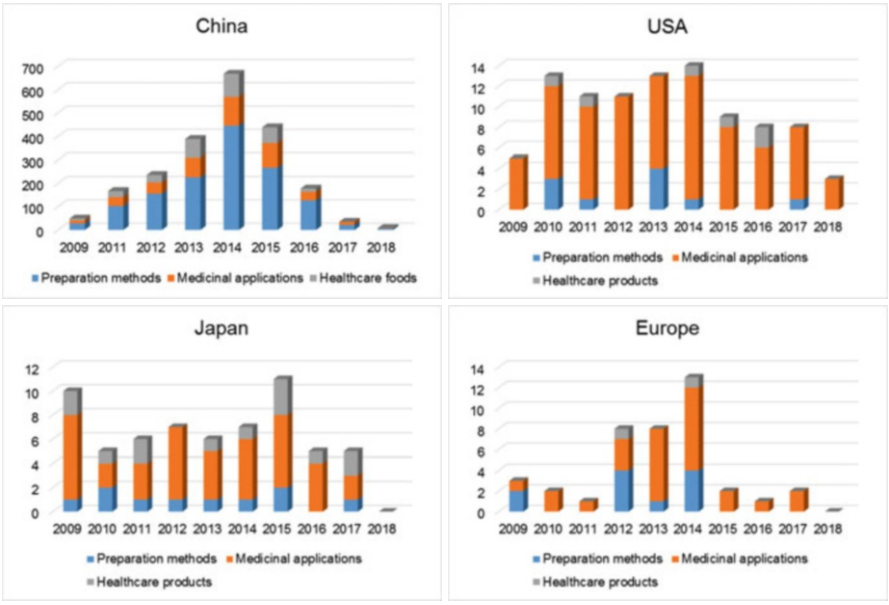


Fig. 2 The trends of the approved patents for different applications (2009.01.01–2018.12.31) in China, the USA, Japan, and Europe

On the other hand, although garlic is a traditional vegetable with good safety, some potential adverse reactions of garlic OSCs were observed and reported both in animals and in humans. The underlying toxicological mechanisms for the side effects and toxicities of garlic OSCs need to be further investigated and assessed thus ensuring the safety for their different applications.

38.11 Cross-References

► Antioxidants in Diets and Food
► Isothiocyanates in Food

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Ginsenosides in Diets

39

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Abstract

Ginseng and its active ingredient ginsenosides are valued for their medicinal properties. More than 100 ginsenosides have been identified from the different parts of the plants in the genus *Panax* and during the processing of the plants. Ginsenosides can increase longevity, reduce blood pressure, alleviate diabetes and cardiovascular diseases, and inhibit cancers. Ginseng is widely used to prepare porridge, soup, and tea. Ginseng products range from neutraceuticals and cosmoceuticals to functional food. No adverse effects of consuming *Panax ginseng* or its extract were observed.

Keywords

Ginseng · Ginsenosides · Anticancer · Anti-inflammatory · Functional food · Cosmoceuticals

39.1 Introduction

Ginseng is well-known to be beneficial to human health: reducing pathological symptoms and protecting against potential diseases. The term ginseng usually refers to the species *Panax ginseng* or more specifically to its root. Ginsenosides are the main active ingredients contributing to the health-promoting effects and are commonly used as a marker for the quality control of ginseng products.

39.2 Bioactive Constituents

Ginsenosides, also known as steroid-like saponins, are the major active pharmacological ingredients in ginseng species which include 14 plants (Table 1) in the genus *Panax*. There have been more than 100 ginsenosides identified, where ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 are the most abundant ones found in the roots of raw ginseng, while others are formed by chemical reactions during the processing of ginseng including by oxidation, hydrolysis, and dehydration as well as by the action of bacteria in the gastrointestinal tract on the orally administered ginseng (Shin et al. 2015). Table 2 lists the chemical formula and plant part of the ginsenosides isolated from *Panax ginseng*. Ginsenosides have a four-ring structure with carbohydrate moieties attached. Each ginsenoside has at least two or three hydroxyl (—OH) groups attached to carbon (C)-3, C-6, and/or C-20, which are free or bound to monomeric, dimeric, or trimeric carbohydrates. Stereoisomers exist, characterized by the position of —OH group on C-20.

Based on the chemical structures, ginsenosides can be classified into two groups: protopanaxadiols (PPD; 3b,12b,20-trihydroxydammar-24-ene) and protopanaxatriols (PPT; 3b,6a,12b,20-tetrahydroxydammar-24-ene). PPD-type ginsenosides such as Rb1, Rb2, Rc, Rd, Rg3, and Rh2 have carbohydrate moieties

Table 1 Plants in the genus *Panax*. Scientific name and common name for the plants in the genus *Panax*

Scientific name	Common name
Panax bipinnatifidus Seem	
Panax bipinnatifidus var. *angustifolius*	
Panax bipinnatifidus var. *bipinnatifidus*	
Panax ginseng C. A. Mey.	Asian ginseng, Chinese ginseng, Korean ginseng
Panax japonicus (T. Nees) C. A. Mey.	Japanese ginseng
Panax notoginseng (Burkill) F. H. Chen	Sanchi
Panax pseudoginseng Wall.	
Panax quinquefolius L.	American ginseng
Panax sokpayensis Shiva K. Sharma & Pandit	
Panax stipuleanatus H. T. Tsai & K. M. Feng	
Panax trifolius L.	
Panax vietnamensis Ha & Grushv.	
Panax wangianus S. C. Sun	
Panax zingiberensis C. Y. Wu & Feng	

attached to C-3, while PPT-type ginsenosides including Re, Rf, Rg1, Rg2, and Rh1 have carbohydrate moieties attached to C-6 belonging to PPT group (Fuzzati 2004). Pseudoginsenoside F11, a ginsenoside isolated from the roots and leaves of *P. quinquefolius* but not found in *P. ginseng*, also belongs to PPT group even though the carbon chain at the 20-position is replaced by a tetrahydrofuran ring. Figure 1 shows the structures of PPD- and PPT-type ginsenosides from *Panax ginseng*.

39.3 Bioavailability and Metabolism

Most ginsenosides are biosynthesized from 2,3-oxidosqualene which forms cycloartenol, dammarenediol-II, and β-amyrin through three different pathways and then transformed into β-sitosterol, dammarane-type saponins, and oleanane-type saponins, respectively. Dammarenediol-II is hydroxylated to generate PPD and is further converted to various saponins by O-glycosylation of PPD that involves the attachment of saccharides to C-3 and/or C-20. On the other hand, PPD may be further hydroxylated to form PPT and biotransformed to various saponins through O-glycosylation of PPT that involves the attachment of saccharides to C-6 and/or C-20. Ginsenosides Rb1, Rb2, Rc, and Rd are typical PPD found in the roots, flower buds, and leaves of *P. ginseng*, whereas Re and Rg1 are typical PPT. Dammarane-type saponins account for a significant portion of saponins in ginseng species; nevertheless, oleanane-type saponins except for ginsenoside Ro are rare.

Comparing *P. ginseng* and *P. quinquefolius*, the contents of ginsenosides Rb2, Rc and Rg1 are higher in *P. ginseng* than in *P. quinquefolius* while Rb1, Rd, and Re are higher in *P. quinquefolius* than in *P. ginseng*. Both Rb1 and Rg1 promote

Table 2 Ginsenosides from *Panax ginseng*. Chemical formula and plant part of the ginsenosides isolated from *Panax ginseng*

No.	Ginsenoside	Formula	Plant part/process
1	Protopanaxadiol	$C_{30}H_{52}O_3$	Hydrolysis
2	Ginsenoside F2	$C_{42}H_{72}O_{13}$	Leaves
3	Ginsenoside Ra1	$C_{58}H_{98}O_{26}$	Roots
4	Ginsenoside Ra2	$C_{58}H_{98}O_{26}$	Roots
5	Ginsenoside Ra3	$C_{59}H_{100}O_{26}$	Roots
6	Ginsenoside Rb1	$C_{54}H_{92}O_{23}$	Roots
7	Ginsenoside Rb2	$C_{53}H_{90}O_{22}$	Roots
8	Ginsenoside Rb3	$C_{53}H_{90}O_{22}$	Roots
9	Ginsenoside Rc	$C_{53}H_{90}O_{22}$	Roots
10	Ginsenoside Rd	$C_{48}H_{82}O_{18}$	Roots
11	Ginsenoside Rg3	$C_{42}H_{72}O_{13}$	Steamed roots
12	Ginsenoside Rh2	$C_{36}H_{62}O_8$	Steamed roots
13	Ginsenoside Rs1	$C_{55}H_{92}O_{23}$	Roots
14	Ginsenoside Rs2	$C_{55}H_{92}O_{23}$	Roots
15	Ginsenoside Rs3	$C_{44}H_{74}O_{14}$	Steamed roots
16	Malonylginsenoside Ra3	$C_{62}H_{102}O_{30}$	Roots
17	Malonylginsenoside Rb1	$C_{57}H_{94}O_{26}$	Roots
18	Malonylginsenoside Rb2	$C_{56}H_{92}O_{25}$	Roots
19	Malonylginsenoside Rc	$C_{56}H_{92}O_{25}$	Roots
20	Malonylginsenoside Rd	$C_{51}H_{84}O_{21}$	Roots
21	Malonylnotoginsenoside R4	$C_{62}H_{102}O_{30}$	Roots
22	Protopanaxatriol	$C_{30}H_{52}O_4$	Hydrolysis
23	Floralginsenoside M	$C_{53}H_{90}O_{22}$	Flower buds
24	Floralginsenoside N	$C_{53}H_{90}O_{22}$	Flower buds
25	Floralginsenoside P	$C_{53}H_{90}O_{23}$	Flower buds
26	Ginsenoside F1	$C_{36}H_{62}O_9$	Leaves
27	Ginsenoside F3	$C_{41}H_{70}O_{13}$	Leaves
28	Ginsenoside Re	$C_{48}H_{82}O_{18}$	Roots
29	Ginsenoside Rf	$C_{42}H_{72}O_{14}$	Roots
30	Ginsenoside Rg1	$C_{42}H_{72}O_{14}$	Roots
31	Ginsenoside Rg2	$C_{42}H_{72}O_{13}$	Roots
32	Ginsenoside Rh1	$C_{36}H_{62}O_9$	Steamed roots
33	20-Glucoginsenoside Rf	$C_{48}H_{82}O_{19}$	Roots
34	Floralginsenoside H	$C_{50}H_{84}O_{21}$	Flower buds
35	Floralginsenoside Tc	$C_{53}H_{90}O_{24}$	Flower buds
36	Floralginsenoside Td	$C_{53}H_{90}O_{24}$	Flower buds
37	Ginsenoside I	$C_{48}H_{82}O_{20}$	Flower buds
38	Ginsenoside II	$C_{48}H_{82}O_{20}$	Flower buds
39	Floralginsenoside A	$C_{42}H_{72}O_{16}$	Flower buds
40	Floralginsenoside C	$C_{41}H_{70}O_{15}$	Flower buds
41	Floralginsenoside J	$C_{48}H_{82}O_{20}$	Flower buds
42	Floralginsenoside Ka	$C_{36}H_{62}O_{11}$	Flower buds

(continued)

Table 2 (continued)

No.	Ginsenoside	Formula	Plant part/process
43	Ginsenoside SL1	$C_{36}H_{62}O_{11}$	Steamed leaves
44	Ginsenoside Rg7	$C_{36}H_{60}O_9$	Leaves
45	Floralginsenoside La	$C_{48}H_{82}O_{19}$	Flower buds
46	Floralginsenoside Lb	$C_{48}H_{82}O_{19}$	Flower buds
47	Floralginsenoside Ta	$C_{36}H_{60}O_{10}$	Flower buds
48	Floralginsenoside E	$C_{42}H_{72}O_{15}$	Flower buds
49	Floralginsenoside F	$C_{42}H_{72}O_{15}$	Flower buds
50	Floralginsenoside G	$C_{50}H_{84}O_{21}$	Flower buds
51	Floralginsenoside K	$C_{48}H_{82}O_{21}$	Flower buds
52	Floralginsenoside O	$C_{53}H_{90}O_{22}$	Flower buds
53	Floralginsenoside B	$C_{42}H_{72}O_{16}$	Flower buds
54	Floralginsenoside D	$C_{41}H_{70}O_{15}$	Flower buds
55	Floralginsenoside I	$C_{48}H_{82}O_{20}$	Flower buds
56	Ginsenoside Rh6	$C_{36}H_{62}O_{11}$	Leaves
57	Ginsenoside ST2	$C_{36}H_{62}O_{10}$	Steamed leaves
58	Ginsenoside Ki	$C_{36}H_{62}O_{10}$	Leaves
59	Ginsenoside Km	$C_{36}H_{62}O_{10}$	Leaves
60	Floralginsenoside Kb	$C_{45}H_{76}O_{19}$	Flower buds
61	Floralginsenoside Kc	$C_{45}H_{76}O_{20}$	Flower buds
62	Floralginsenoside Tb	$C_{35}H_{62}O_{11}$	Flower buds
63	25-Hydroxyprotopanaxadiol	$C_{30}H_{54}O_4$	Fruits
64	25-Hydroxyprotopanaxatriol	$C_{30}H_{54}O_5$	Fruits
65	Dehroxyprotopanaxadional I	$C_{30}H_{50}O_2$	Steamed roots
66	Ginsenoside Rg5	$C_{42}H_{70}O_{12}$	Steamed roots
67	Ginsenoside Rh3	$C_{36}H_{60}O_7$	Steamed roots
68	Ginsenoside Rs4	$C_{44}H_{72}O_{13}$	Steamed roots
69	Dehroxyprotopanaxatriol I	$C_{30}H_{50}O_3$	Steamed roots
70	Ginsenoside F4	$C_{42}H_{70}O_{12}$	Roots
71	Ginsenoside Rh4	$C_{36}H_{60}O_8$	Steamed roots
72	Ginsenoside Rs6	$C_{38}H_{62}O_9$	Steamed roots
73	Ginsenoside Rz1	$C_{42}H_{70}O_{12}$	Steamed roots
74	Dehroxyprotopanaxadional II	$C_{30}H_{50}O_2$	Steamed roots
75	Ginsenoside Rk1	$C_{42}H_{70}O_{12}$	Steamed roots
76	Ginsenoside Rk2	$C_{36}H_{60}O_7$	Steamed roots
77	Ginsenoside Rs5	$C_{44}H_{72}O_{13}$	Steamed roots
78	Dehroxyprotopanaxatriol II	$C_{30}H_{50}O_3$	Steamed roots
79	Ginsenoside Rg6	$C_{42}H_{70}O_{12}$	Steamed roots
80	Ginsenoside Rk3	$C_{36}H_{70}O_8$	Steamed roots
81	Ginsenoside Rs7	$C_{38}H_{62}O_9$	Steamed roots
82	Panaxadiol	$C_{30}H_{52}O_3$	Hydrolysis
83	Panaxatriol	$C_{30}H_{52}O_4$	Hydrolysis
84	Ginsenoside Rh9	$C_{36}H_{60}O_9$	Leaves
85	12,23-Epoxyginsenoside Rg1	$C_{42}H_{70}O_{14}$	Leaves

(continued)

Table 2 (continued)

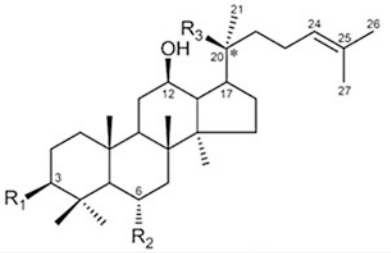
No.	Ginsenoside	Formula	Plant part/process
86	Panaxadione	$C_{30}H_{48}O_5$	Seeds
87	Ginsenoside Rh5	$C_{36}H_{60}O_9$	Steamed roots
88	Ginsenoside Rh7	$C_{36}H_{60}O_9$	Leaves
89	Ginsenoside Rh8	$C_{36}H_{60}O_9$	Leaves
90	Ginsenoside Ro	$C_{48}H_{76}O_{19}$	Roots
91	Ginsenoside SL2	$C_{42}H_{70}O_{14}$	Steamed leaves
92	Ginsenoside ST1	$C_{36}H_{60}O_{10}$	Steamed leaves
93	Ginsenoside SL3	$C_{42}H_{70}O_{14}$	Steamed leaves
94	Hexanordammaran	$C_{24}H_{40}O_4$	Leaves
95	Isoprotopanaxadiol	$C_{30}H_{52}O_3$	Hydrolysis

central nervous system (CNS) activities, but the effect of Rg1 is more potent. Therefore, *P. ginseng* with higher amount of Rg1 is a stimulant, while *P. quinquefolius* with more Rb1 is calming to CNS (Chen et al. 2008).

Some ginsenosides are formed from the peroxidation of PPD or PPT, mostly at or around the double bond between C-24 and C-25 with a hydroperoxyl group at C-26 or C-27, which can be reduced to hydroxyl group (Niki et al. 2005). Besides, some ginsenosides have a hydroperoxyl or hydroxyl group at C-24 and a double bond between C-25 and C-26. Some ginsenosides with a double bond between C-23 and C-24 have a hydroperoxyl or hydroxyl group at C-25. Regarding the possible geometric isomerism in compounds with a double bond between C-23 and C-24, the (*E*)-form isomers are more common than the (*Z*)-form.

The oxidative cleavage of the double bond of PPD, 23-hydroxyprotopanaxadiol, and PPT leads to the formation of an aldehyde with three fewer C atoms, yielding 3β,12β,20-trihydroxy-25,26,27-trinordammar-24-al (floralginsenoside Kb), 3β,12β,20,23-tetrahydroxy-25,26,27-trinordammar-24-al (floralginsenoside Kc), and 3β,6α,12β,20-tetrahydroxy-25,26,27-trinordammar-24-al (floralginsenoside Tb), respectively. The hydration of the double bond of PPD and PPT generates 25-hydroxyprotopanaxadiol and 25-hydroxyprotopanaxatriol, respectively. Steamed or heat processed, PPD- and PPT-type ginsenosides are deglycosylated and dehydrated at C-20 to form double bond either between C-20 and C-21 or between C-20 and C-22, where geometric isomerism presents in the latter case. The acid hydrolysis of PPD- and PPT-type ginsenosides result in the production of a six-membered ring containing oxygen, forming 3β,12β-dihydroxy-20,25-epoxydammarane (panaxadiol) and 3β,6α,12β-trihydroxy-20,25-epoxydammarane (panaxatriol). In addition, there are derivatives of 3β,6α,20-dihydroxy-12,23-epoxydrammar-24-ene or 6α,25-dihydroxy-20,24-epoxydammar-3,12-dione with the epoxy group present between C-12 and C-23 and between C-20 and C-24, respectively.

Ginsenosides are mostly metabolized by intestinal bacteria including *Bacteroides*, *Eubacterium*, *Fusobacterium*, and *Prevotella* when taken orally. PPD-type ginsenosides are deglycosylated at C-3 and PPD or compound K may



Ac: acetyl
Ara(fur): α-L-arabinofuranosyl
Ara(pyr): α-L-arabinopyranosyl
Glc: β-o-glycopyranosyl
Ma: malonyl
Rha: α-L-rhamnopyranosyl
Xyl: β-o-xylopyranosyl

		R_1	R_2	R_3
1*	protopanaxadiol	OH	H	OH
2	ginsenoside F2	OGlc	H	OGlc
3	ginsenoside Ra1	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Ara(pyr)^{4\text{—}1}Xyl$
4	ginsenoside Ra2	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Ara(fur)^{2\text{—}1}Xyl$
5	ginsenoside Ra3	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Glc^{3\text{—}1}Xyl$
6	ginsenoside Rb1	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Glc$
7	ginsenoside Rb2	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Ara(pyr)$
8	ginsenoside Rb3	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Xyl$
9	ginsenoside Rc	$OGlc^{2\text{—}1}Glc$	H	$OGlc^{6\text{—}1}Ara(fur)$
10	ginsenoside Rd	$OGlc^{2\text{—}1}Glc$	H	OGlc
11*	ginsenoside Rg3	$OGlc^{2\text{—}1}Glc$	H	OH
12	ginsenoside Rh2	OGlc	H	OH
13	ginsenoside Rs1	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Ara(pyr)$
14	ginsenoside Rs2	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Ara(fur)$
15*	ginsenoside Rs3	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	OH
16	malonylginsenoside Ra3	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Glc^{3\text{—}1}Xyl$
17	malonylginsenoside Rb1	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Glc$
18	malonylginsenoside Rb2	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Ara(pyr)$
19	malonylginsenoside Rc	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Ara(fur)$
20	malonylginsenoside Rd	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	OGlc
21	malonylnotoginsenoside R4	$OGlc^{2\text{—}1}Glc^{6}Ac$	H	$OGlc^{6\text{—}1}Glc^{6\text{—}1}Xyl$
22*	protopanaxatriol	OH	OH	OH
23	floralginsenoside M	OH	$OGlc^{2\text{—}1}Rha$	$OGlc^{6\text{—}1}Ara(fur)$
24	floralginsenoside N	OH	$OGlc^{2\text{—}1}Rha$	$OGlc^{6\text{—}1}Ara(pyr)$
25	floralginsenoside P	$OGlc^{2\text{—}1}Glc$	OH	$OGlc^{6\text{—}1}Ara(pyr)$
26	ginsenoside F1	OH	OH	OGlc
27	ginsenoside F3	OH	OH	$OGlc^{6\text{—}1}Ara(pyr)$
28	ginsenoside Re	OH	$OGlc^{2\text{—}1}Rha$	OGlc
29	ginsenoside Rf	OH	$OGlc^{2\text{—}1}Glc$	OH
30	ginsenoside Rg1	OH	OGlc	OGlc
31*	ginsenoside Rg2	OH	$OGlc^{2\text{—}1}Rha$	OH
32*	ginsenoside Rh1	OH	OGlc	OH
33	20-glucoginsenoside Rf	OH	$OGlc^{2\text{—}1}Glc$	OGlc

Fig. 1 Chemical structures of protopanaxadiol- and protopanaxatriol-type ginsenosides from *Panax ginseng*. ∗1, 11, 15, 22, 31, 32 illustrating the presence of (S)- and (R)-configuration at C-20. Ac, acetyl; Ara(fur), α-L-arabinofuranosyl; Ara(pyr), α-L-arabinopyranosyl; Glc, β-o-glycopyranosyl; Ma, malonyl; Rha, α-L-rhamnopyranosyl; Xyl, β-o-xylopyranosyl

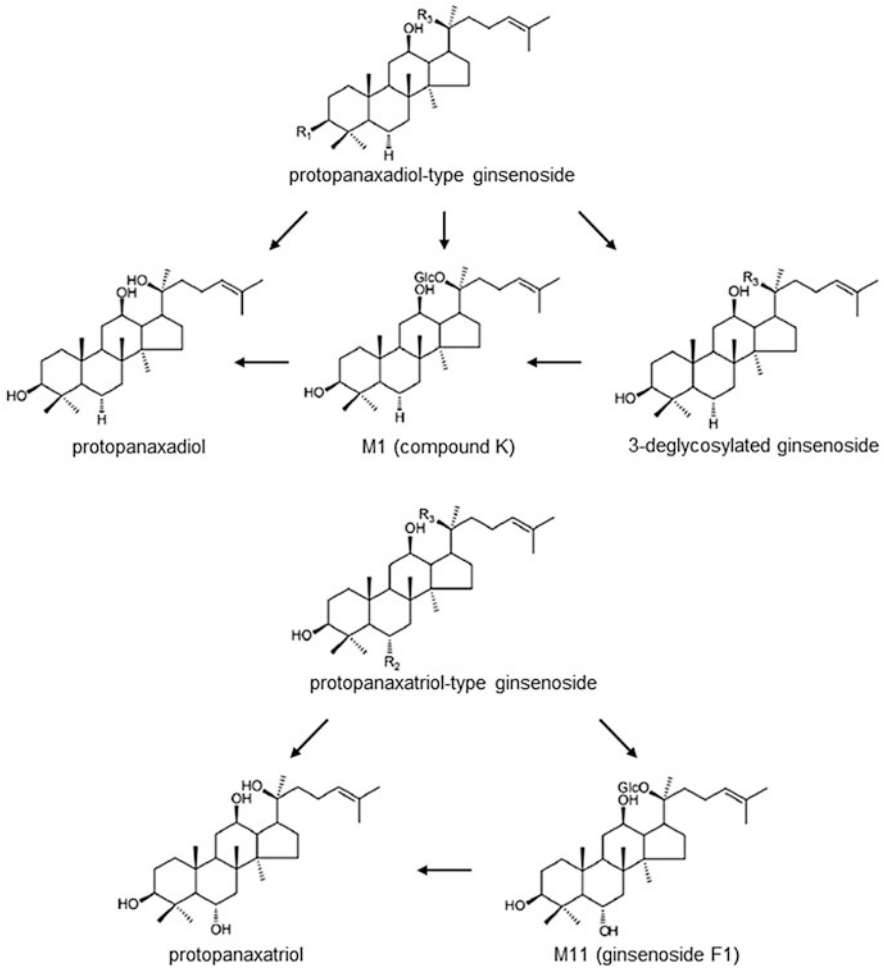


Fig. 2 Metabolic pathways of protopanaxadiol- and protopanaxatriol-type ginsenosides by intestinal bacteria

result, while PPT-type ginsenosides are deglycosylated at C-6 or C-20 and transformed to PPT or ginsenoside F1 (Fig. 2).

39.4 Bioactivities

39.4.1 Anti-inflammatory Effects

A number of ginsenosides have been demonstrated to exhibit anti-inflammatory effects. Ginsenoside Rb1 and its bacterial metabolite compound K also suppress the activations of nuclear factor-kappa B (NF-κB), IL-1 receptor-associated kinase

(IRAK)-1, IκB kinase-α (IKK-α), and mitogen-activated protein kinases (MAPKs) in lipopolysaccharide (LPS)-induced murine peritoneal macrophages and in 2,4,6-trinitrobenzene sulfuric acid (TNBS)-induced colitis animal model (Joh et al. 2011). Likewise, compound K inhibits intestinal inflammation by negatively regulating NF-κB signaling in dextran sulfate sodium-induced colitis animal model (Li et al. 2014a). Ginsenosides Rb2 and Rd inhibit NF-κB activation and tumor necrosis factor-alpha (TNF-α) expression, having neuroprotective effects in LPS-induced N9 microglial cells (Wu et al. 2007). Furthermore, ginsenoside Rd exerts neuroprotective effects in rats with transient focal cerebral ischemia through suppression of oxidative stress and inflammatory responses (Ye et al. 2011b). Ginsenosides Rb2 exhibits anti-inflammatory effects by decreasing the expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and NF-κB activity in LPS-stimulated RAW264.7 cells and mouse liver (Kim et al. 2013a).

Ginsenoside Re lowers the expressions of interleukin-1β (IL-1β) and TNF-α, as well as NF-κB activation to protect against LPS-induced inflammation on macrophages and in TNBS-induced colitis mouse model (Lee et al. 2012). Ginsenoside Rg1 decreases the expressions of iNOS, COX-2, TNF-α, IL-1β, and NF-κB in LPS-treated BV-2 microglial cells (Zong et al. 2012) and suppresses NF-κB signal pathway in both LPS-stimulated RAW264.7 cells and mouse peritoneal macrophages (Wang et al. 2014b). Consistent with the in vitro results, ginsenoside Rg1 ameliorates alcoholic hepatitis (Gao et al. 2015) and TNBS-induced colitis (Lee et al. 2015) in animal models through suppressing NF-κB activation. Modulating inflammatory response and apoptosis, ginsenoside Rb1 protects from ischemia/reperfusion (IR) injury in livers from mice (Guo et al. 2011; Tao et al. 2014). Daily administration of ginsenoside Rg3 for 3 weeks alleviates LPS-triggered learning and memory impairments by inhibiting the inflammation in the rat hippocampus (Lee et al. 2013). Ginsenoside Rg5 has been shown to attenuate lung inflammation by suppressing NF-κB, TNF-α, IL-1β, iNOS, and COX-2 in LPS-stimulated alveolar macrophages and in the bronchoalveolar lavage fluid from LPS-injected mice (Kim et al. 2012).

Ginsenoside Rh1 has been demonstrated to exert anti-inflammatory effects: downregulating the expressions of COX-2 and iNOS in IFN-γ-treated BV2 microglial cells (Jung et al. 2010) and diminishing the serum levels of IgE and IL-6 in mice with atopic dermatitis (Zheng et al. 2011). In addition, ginsenoside Rh2 inhibits the activation of NF-κB and p38 MAPK in murine asthma model (Li et al. 2015).

39.4.2 Antidiabetic and Cardiovascular Effects

Ginsenoside Rb1 ameliorates homocysteine (Hcy)-induced endothelial dysfunction and oxidative stress in porcine coronary arteries (Zhou et al. 2005) and suppresses H_2O_2-induced senescence of human endothelial cells through eNOS-dependent mechanism (Liu et al. 2012). Ginsenoside Rb3 ameliorates isoproterenol-induced myocardial injury and heart function impairment in Sprague-Dawley (SD) rats through inhibition of oxidative stress (Wang et al. 2010a) and protects

cardiomyocytes against ischemia/reperfusion injury by suppressing JNK-mediated NF-κB pathway (Ma et al. 2014). Moreover, ginsenoside Rb3 decreases angiotensin (Ang) II-induced vascular smooth muscle cell (VSMC) proliferation (Wang et al. 2010b). Ginsenoside Rb3 exerted antidiabetic effects in alloxan-induced diabetic mice (Bu et al. 2012). Ginsenoside Rb3 also restores impaired endothelial function in renal arteries from spontaneously hypertensive rats ex vivo through suppression of oxidative stress and improvement of NO bioavailability (Wang et al. 2014a).

Ginsenosides Rb1 and Rg1 induce vasodilatation in mouse coronary arteries (Pan et al. 2012). Ginsenoside Rg3 administration improves islet function before islet transplantation and ameliorates both cytokine-induced damage and apoptosis in Balb/c mice (Kim et al. 2014). Ginsenosides Rg6, F4, Rk3, and Rh4 inhibit U46619-induced platelet aggregation (Lee et al. 2010a). Ginsenoside Re triggers NO production in human endothelial cells (Leung et al. 2007). Saponin extract from *Panax japonicus* alleviates myocardial infarction in SD rats through inhibition of NF-κB and MAPK signaling pathways with increased SIRT1 expression (Wei et al. 2014). Notoginsenoside R1, a ginsenoside found in *P. notoginseng*, inhibits hypoxia- and hypercapnia-induced vasoconstriction in isolated rat pulmonary arteries by decreasing ERK expression (Xu et al. 2014).

39.4.3 Anticancer Effects

Ginsenoside Rg3 exerts inhibitory effects to different cancer cells, for example, inhibiting the proliferation of prostate cancer cells (Peng et al. 2019) and suppressing the migration and invasion of liver cancer cells (Sun et al. 2019). Ginsenoside Rg5 suppresses cell proliferation and triggers apoptosis in retinoblastoma cells through inactivation of AKT pathway and downregulation of BCL2 expression (Cui et al. 2018) and protects against breast cancer by inducing apoptosis and autophagy in mice (Liu and Fan 2018). Ginsenoside Rh2 inhibits proliferation in lung cancer cells (Ge et al. 2017) as prostate cancer cell growth (Gao and Zheng 2018). Ginsenoside Rk3 has been shown to possess anti-esophageal cancer activity in the KYSE150 xenograft model (Liu et al. 2019). 25-Hydroxyprotopanaxadiol (25-OH-PPD) found in the ginseng fruit inhibits cell growth and proliferation in human prostate cancer cells (LNCaP and PC3 cells) as well as the tumor growth in a mouse PC3 xenograft tumor model (Wang et al. 2008). Ginsenoside compound reduces growth of lung cancer cells (Chen et al. 2019). Other ginsenosides like Rb2, Rd, Rh4, and Rk1 exert anticancer effects.

39.4.4 Potential Role in Neurodegenerative Disorders

Ginsenoside Rb1 inhibits local inflammation after cerebral ischemia in Sprague-Dawley rats (Zhu et al. 2012). Ginsenoside Rb1 also ameliorates cerebral IR injury by activating peroxisome proliferator-activated receptor-γ (PPARγ)/heme

oxygenase-1 (HO-1) (Yang et al. 2015) or by suppressing protease-activated receptor-1 (PAR-1) expression in rats (Xie et al. 2015). Ginsenoside Rd improves neuronal function in aged mice with transient middle cerebral artery occlusion (MCAO) (Ye et al. 2011a). Ginsenoside Rg1 induces neuroprotection in transgenic Alzheimer's disease mice (Fang et al. 2012). Furthermore, ginsenoside Rg5 suppresses acetylcholinesterase (AChE) activity and the expressions of COX-2 and iNOS while upregulates the expressions of insulin-like growth factor-1 (IGF-1) and brain-derived neurotrophic factor (BDNF) to improve cognitive function in streptozotocin (STZ)-induced Alzheimer's disease (AD) rats (Chu et al. 2014). Likewise, ginsenoside Rg5 and its metabolite ginsenoside Rh3 inhibit AChE activity and elevate BDNF expression and cAMP response element-binding protein (CREB) phosphorylation, reversing scopolamine-induced memory deficit in mice (Kim et al. 2013b).

39.4.5 Antioxidant Treatment and Alcoholism

Ginsenoside Rg1 exerts protective action against hepatic fibrosis induced by alcohol and CCl4 and antioxidant and anti-inflammatory effects in rat through upregulating Nrf2 expression (Li et al. 2014b). Ginsenosides Rg3 and Rh2 from Korean red ginseng (*P. ginseng*) suppress alcohol-induced oxidative injury through inhibition of MAPK pathway in mouse hepatocytes in vitro (Park et al. 2012).

39.5 Benefits

The PPT-rich ginseng extract (Ginseol K-g1) from *P. ginseng* is composed of ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 and is shown to decrease blood pressure after 4 weeks of treatment in a clinical study involving 90 subjects of age $>$ 20 years old with prehypertension or stage I hypertension (Rhee et al. 2014). Likewise, ginsenoside Rg3-enriched Korean red ginseng reduces central and peripheral arterial pressures in healthy individuals (Jovanovski et al. 2014a). In a randomized controlled trial including 16 healthy individual, the ginsenoside extract of Korean red ginseng (steamed *P. ginseng*) improves flow-mediated dilatation (Jovanovski et al. 2014b). North American ginseng extract lowers the DNA damage in lymphocytes obtained from healthy individuals at 90 min postirradiation (Lee et al. 2010b).

39.6 Application in Food (Inc. Correctly Cooking Food Rich in Phytochemicals)

As ginseng is valued for health benefits in various cultures globally, preparation and right cooking techniques play an important role in preserving the active phytochemical to be consumed. The Koreans has associated ginseng for centuries

to restore stamina; to increase longevity; to treat high blood pressure, diabetes, and cholesterol; and to be used as aphrodisiac. Being one of the largest producers of ginseng, Koreans enjoys the ginseng root as spice in food and tea.

The Samgye-tang, the Korean ginseng porridge, is prepared traditionally by soaking the glutinous rice in water for 2 h while cleaning the chicken by rubbing kosher salt before being rinsed under running water. Approximately, 5 cm of fresh ginseng should be silted to release its pungent flavor during cooking. Then, the ginseng root, red dates, garlic, cloves, and the soaked rice are stuffed into the chicken cavity. The feet of the chicken is tied with kitchen twine and placed in a soup pot filled with water. All these ingredients were left to simmer up to an hour before being enjoyed.

As Samgye-tang is widely enjoyed as Korean ginseng cuisine, in the west, ginseng chicken soup is famous among these traditional herb lovers. It is believed ginseng stores energy and promotes vitality. The ginseng chicken soup is prepared by blanching the chicken pieces in boiling hot water for 5 min and drained. Then, about 40 g of American ginseng, red dates, and the blanched chicken are placed in a pot and boiled over high heat for 10 min. The heat temperature is then lowered to reduce flame and to simmer for 40 min before wolfberries are being added. All these ingredients have to be cooked for another 20 min and then salt is added to taste.

Besides cuisine, ginseng is also made into beverages, being enjoyed mainly by the Chinese community. The ginseng chrysanthemum tea is prepared by boiling 50 g of chrysanthemum flower in a pot at reduced heat and by simmering for less than a minute. The flower is then strained. About 5 g of ginseng is added and simmered for another 5 min before the remainder of the ginseng is being strained. Rock sugar is added to taste and slowly heated until the sugar is dissolved. The tea is then cold to be served.

Insam-cha is another famous ginseng-based tea from Korea. This tea is prepared by peeling and slicing the ginseng into 7–8 thin slices. The ginseng is then coated with a generous amount of honey and let to rest for 15 min. Hot water is then poured over the prepared ginseng and let to steep for 10 min. The ginseng slices are then strained and highly aromatic tea is served at room temperature.

Moreover, the Canadian ginseng tea is prepared by adding 20 g of Canadian ginseng slices and then cleaned and placed in tea bag. In 1.5 liters of water, 20 g of chrysanthemum and the Canadian ginseng is simmered for 30 min before adding 1 tablespoon of soaked wolfberries. These ingredients are then simmered for 5 min before rock sugar is added to taste. The tea is enjoyed chilled or at room temperature.

39.7 Safety: Toxicity and Side Effects

The safety of ginseng consumption had been a topic of research interest since the 1970. For years, researchers had executed the in vivo and in vitro studies pertaining to toxicity and safety of ginseng around the world. Siegel (1979) had coined on “ginseng abuse syndrome.” He reported the undesired effect of ginseng

consumption on 133 patients with constant ginseng consumption up to 2 years. Majority of the correspondent experienced morning diarrhea (35%), skin eruption (25%), nervousness (25%), sleeplessness (20%), hypertension (17%), edema, decreased appetite, depression, and hypotension (10%). His research also concluded that the undesirable side effects manifested in prolonged consumption of high dose of ginseng. It is known that ginsenosides especially ginsenoside Rb1 and ginsenoside Rg1 from *Panax ginseng* display a variety of pharmacological and therapeutic effects on central nervous system (CNS) disorders and metabolic-related diseases such as cardiovascular disease, endocrine secretions, aging, and immune functions (Park et al. 2013; Ernst 2010). Through animal-based assay, Chan and co-workers (2011) had assessed the carcinogenic potential of *Panax ginseng* for 2 years under the US National Toxicology Program (NTP) which revealed that consumption of *Panax ginseng* is neither toxic nor tumorigenic. Park and co-workers (2013) had also studied the pharmacokinetics and bioavailability of ginsenosides based on animal model. Groups of male and female Sprague-Dawley rats were fed with extract of Korean red ginseng and *Panax ginseng* at various concentrations (0, 500, 1000, and 2000 mg/kg/day) for 4 weeks. This experiment revealed that there is no death or clinical abnormalities observed on experimental animal at highest dose of ginseng extracts administered.

Park and co-workers (2013) conducted 4 weeks of oral toxicity studies in rats to observe whether consumptions of Korean red ginseng could induce toxicity to humans. Groups of male and female Sprague-Dawley rats were fed with *Panax ginseng* extract with dose levels of 0, 500, 1,000, and 2,000 mg/kg/day for 4 weeks, orally. Overall, there were no deaths or clinical abnormalities observed among the experimental animal, and the results revealed that no observed adverse effect level of Korean red ginseng extract was established at 2,000 mg/day.

39.8 Marketed Products

As treasure for many reasons, products developed based on ginseng range from neutraceuticals and cosmoceuticals to functional food. As ginseng is rich in phytochemicals and antioxidant, it is believed to elevate mental and physical activities, to combat fatigue, to stimulate alertness, and to reduce signs of skin aging.

39.8.1 Ginseng-Based Beverages

Many organic energy booster formula incorporated *Panax ginseng*, to name a few, Ginseng Energy™ drink from Pakistan, Monster Taurine™ and ginseng energy drink from the United States, Orka Icelandic Ginseng Energy™ drink from Iceland, Elcro Red Ginseng Plus™, and Root9™ red ginseng drink from Korea. Variety of ginseng-based tea infused with lavender, chrysanthemum, and chamomile is enjoyed in many Asian countries as “vitality drink,” which is an alternative to energy drink.

39.8.2 Ginseng-Based Supplements

As ginseng is believed to elevate immune system, boost memory power, promote relaxation, and manage sexual dysfunction in men, various products were formulated to meet this demand, globally in many forms, from powder, capsules, and even droplets. To name a few brands, Yours Nutrition Korean Red Ginseng™ is formulated with concentrated ginsenosides in capsule form at 10% to boost immunity and energy and significantly reduce levels of stress. The Auragin Korean Ginseng™ is a supplement produced in Korea with each capsule containing 300 mg of 8% ginsenosides as active ingredient. The NuSci Panax Ginseng™ is formulated in powder form with recommended serving containing 12% of ginsenosides. Nature's Answer American Ginseng™ is formulated in liquid form and to be used as droplets. It is potent as it contains 2000 mg of ginseng extract constituting 75% of ginsenosides. It is recommended that a few drops of this liquid are needed to be diluted in a glass of water before consuming. The Solgar Korean Ginseng™ comes in capsule form containing 250 mg of Korean ginseng extract (8% ginsenosides).

39.8.3 Ginseng-Based Cosmoceuticals

The range of cosmoceutical products formulated based on ginseng roots are on rise globally. The *Panax ginseng* root extract contains high concentrations of phytonutrients that help to fight the effects of free radicals due to sun exposure and environmental pollution. As the science develop which can fight the effects of sun-induced free radicals from exposure to sunlight and environmental pollution. Ginseng root extract has anti-aging properties, helps to reduce the appearance of wrinkles, reduces collagen degradation induced by stress and environmental factors, brightens skin complexion, reduces dark spots, and protects the skin from solar UVB radiation (Kang et al. 2009; Hwang et al. 2017). This property is treasured as agents for modern age skincare products.

Among famous ginseng-based skincare brand is Sulwhasoo™, the Korean skincare brand had created the world first ginseng-based cosmetics, "The ABC Ginseng Cream" as early as 1966 followed by Dr. Pierre™ catering ginseng based exfoliating gel to lotions. While ORIGINS™ had formulated GinZing™, the refreshing ginseng-based eye cream. The NutraChamps Korean Panax Ginseng™ is a vegetable-based capsule with rice flour as filler containing 5% of ginsenosides. This product is formulated for stress-calming and acne-clearing purposes. The aSquared Nutrition™ capsules are formulated using *Panax ginseng* which comes in a capsule of 500 mg. It is believed that aSquared Nutrition™ capsules could prevent common cold and improve mental arithmetic and are a top quality ginseng supplement for acne.

39.9 Patents

According to the World Intellectual Property Organization (WIPO), a patent is an exclusive right granted for an invention of a product or a process that provides a new way of doing something or offers a new technical solution to a problem. The patent

is granted when the technical information pertaining to the invention is disclosed to the public upon patent application. Currently, the application patent rights regarding ginseng-based products or methods pertaining to ginsenoside extraction from various ginseng species is widely available and on increasing trend. Among the registered patent rights found globally is stated in the Table 3.

Table 3 Patent application on ginseng-based products or ginsenoside extraction methods

Year	Patent ID	Country issued	Product or method patented
1979	US4157894A	United States	Production and analysis of ginseng root extract
1987	US5071839A	United States	Safe pharmaceutical composition for decreasing side effects of antiviral drugs and increasing the immune function (SDI) II
1993	US5230889A	United States	Enriched nutritious food product comprising powder of ginseng and method for producing powder of ginseng
2001	EP0831864A1	Korea	Processed ginseng having enhanced pharmacological effect
2001	US6326202B1	United States	Stable high ginsenoside-yielding callus line of *Panax quinquefolius* (American ginseng) and a method for developing such stable high ginsenoside-yielding callus line
2003	US20030077341A1	United States	Processes of making North American ginseng fractions, products containing them, and use as immunomodulators
2004	WO2005070235A1	Korea	A drink containing ultrafine powder of ginseng and the method thereof
2005	WO2005120535A1	Korea	Composition comprising ginsenosides for treating or preventing angiostenosis and restenosis
2007	CN1656949A	China	Five-ginseng health-care cigarette
2000	WO2000064278A1	Japan	Ginseng berry health products
2008	US7824716B1	United States	Herbal women's health formula.
2010	US8337821B2	United States	Composition for treatment of hyperhidrosis
2010	CN102139013A	China	Health-care tonic wine for strengthening yang
2010	US20110045129A1	United States	Plant extracts (ginseng species) as additives for yeast-raised baked good
2011	CN102139013ACN102302078A	China	Ginseng soft sweets and production process thereof

(continued)

Table 3 (continued)

Year	Patent ID	Country issued	Product or method patented
2011	WO2011062332A1	Korea	A method for extracting ginsenoside-abundant extract from ginseng by using weak alkali water
2011	CA2480438C	Canada	Novel use of the extract of processed ginseng and saponin isolated therefrom
2011	WO2011043564A2	Korea	Composition containing black ginseng extracts for preventing or treating liver cancer
2012	CN102948750A	China	Stranded *Radix puerariae* pseudo-ginseng *Lilium brownii* capsule
2013	WO2013012180A2	Korea	Production method for a red ginseng liquid concentrate having boosted levels of ginsenoside rg3 and rh2
2013	WO2015029134A1	Japan	Ginsenoide composition is derived from ginseng and particulary useful as a concoction for oral use
2013	CN103082261A	China	Ginseng root condiment manufacturing method
2015	EP2152281B1	Korea	Cosmetic use of ginseng berry extract for skin whitening
2016	CN105815717A	China	*Vaccinium bracteatum* Thunb. fruit flavor red ginseng fruit jam
2017	CN103565679A	China	Fresh ginseng extract preparation method and application of fresh ginseng extract in cosmetics
2017	CN107518401A	China	Red ginseng and blueberry cream and preparation method thereof
2017	CN104593178A	China	Multifunctional handmade soap containing rare ginseng saponins
2019	CN100452995C	China	Active American ginseng health-care beverage and fruit tea

39.10 Perspectives

Ginseng is a one of the key medicinal ingredients in traditional Chinese medicine. Belonging to the family Araliaceae, the name *Panax* was derived from a Greek word meaning “all-healing.” The *Panax* family constitute of various species known species, *Panax ginseng*, *Panax quinquefolius* (American ginseng), *Panax notoginseng*, and *Panax japonicus* (Japanese ginseng). Mainly valued for its roots, these species of ginseng have economic values reaching US$ 300 million globally. Although there is a wide debate and skeptical views on usage and consumption of ginseng, this traditional herb will be treasured for many generations.

39.11 Cross-References

► Antioxidants in Diets and Food
► Saponins in Food

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Procyanidins in Food

40

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Abstract

Proanthocyanidins (PAs) found in fruits, cereals, beans, nuts, and spices, are colorless, oligomeric, and polymeric plant secondary metabolites formed from flavan-3-ol molecules. The complexity of final structures depends on the hydroxylation pattern, and location and stereochemistry of the interflavan linkage between extension and end units. PAs exert a wide range of biological activities (antidiabetic, anticancer, antithrombotic, beneficial in cardiovascular disorders, urinary tract infections, or ADHD) which were well documented in in vitro assays and in in vivo animal models, but also were verified in clinical studies involving human subjects. Interestingly, only 5–10% of the total ingested PAs are absorbed in the small intestine, the majority undergoing microbiota modifications. Hence, the beneficial effects proved for PAs consumption should be rather attributed to metabolites formed by the colonic microbiota. PAs can be recovered as added value products from food industry wastes like cocoa beans, grapes, tea, strawberries, raspberries, nuts, cranberries, cocoa beans, cinnamon, apples, and apricots being produced in millions of tons each year worldwide. The abundance of PAs and their common occurrence in food products make this group of plant secondary metabolites a very valuable reservoir of active principles which contribute to human wellbeing and health.

Keywords

Flavan-3-ol-oligomers and polimers · Extracts and foods rich in procyanidins · Metabolism · Bioactivity · Safety

40.1 Introduction

Proanthocyanidins (PAs, also termed condensed tannins) are colorless, oligomeric, and polymeric plant secondary metabolites formed from flavan-3-ol molecules (Khanbabaee and Van Ree 2001; Santos-Buelga and Scalbert 2000), which can be also substituted by methyl, acyl, galloyl, or glycosyl moieties (He et al. 2008). PAs, being considered the second most abundant group of natural phenolics after lignin (He et al. 2008), were discovered in 1947 by French doctor Jacques Masquelier (Masquelier 1987; Passwater 1991). Masquelier's research on PAs was inspired by finding that crew of famous explorer Cartiers was cured from scurvy with tea made from needles and bark of pine, served by native Canadians (Carper 1998). During his work for PhD thesis, Masquelier extracted a colorless fraction from the red-brown

skin of peanuts and confirmed its biological activity as vitamin P. He also proposed that major components of this fraction are oligomers of flavan-3-ol units (oligomeric procyanidins, OPCs) (Weseler and Bast 2017). OPCs derived from peanut skins were then successfully marketed as a blood vessel protectant, and in 1969, Masquelier obtained a US patent for the extraction of OPCs from pine bark and grape seeds and for its medical application (Masquelier 1987; Passwater 1991). Since that time, PAs were extensively studied in terms of structure elucidation and biological activities and over 200 oligomers with degree of polymerization (DP) up to 5 have been described (He et al. 2008). In plant material OPCs are accompanied by PAs with higher DPs reaching even 30 building blocks in grape seeds or black soybean seed coat (Takahata et al. 2001).

Proanthocyanidins are widely distributed in roots, wood, bark, leaves, fruits, and seeds of many herbaceous and woody plants and, most importantly, in variety of edible plants consumed in a daily diet (Engström et al. 2014). Fruits (apples, apricots, avocado, banana, berries, cherries, dates, grapes, kiwi, mango, pears, plums), cereals and beans (barley, beans, cocoa, sorghum, pea), and nuts and spices (cinnamon and curry) contribute to the everyday intake of PAs. Also, snacks and beverages (chocolate, fruit juices, red wine, and beer) are good sources, especially, of OPCs (Prior and Gu 2005). The most important plant species containing PAs are presented below.

40.1.1 *Pinus pinaster* Aiton (syn. *Pinus maritima* Lam)

Pinus radicata D.Don (syn. *Pinus insignis* Douglas ex Loudon) *Pinus massoniana* Lamb

The bark and leaves of pine species and also of other conifers were used as a natural remedy since ancient times. Pine bark served as a food source in emergencies, while its decoction cured scurvy indicating that some of active ingredients correspond to the action of vitamin C. Application of the bark of spruce, pine, and juniper for wound healing was also known at that time (Li et al. 2015). The bark, needles, pollen, and turpentine of *P. massoniana* have been used in traditional Chinese medicine as a remedy for a treatment of hemorrhages, rheumatism, arthralgia, inflammation, and cancer (Cui et al. 2005). Due to the presence of flavonoids, catechins, phenolic acids, but mainly proanthocyanidins (non-conjugated procyanidins B1 and M1 (formed in vivo from catechin polymer by gut microbiota)) (Jerez et al. 2007), the extract of pine bark has found wide application in the fields of nutrition and health. Over the past few decades, it has been used in poor blood vessel conditions (Li et al. 2015). The procedure of preparation of standardized water extract from pine bark was patented and product obtained from *P. pinaster* was marketed worldwide under the trademark Pycnogenol®, while extract from *P. radiate* was named Enzogenol®. The variety of properties (antioxidant, anti-inflammatory, anticarcinogenic, cardioprotective and neuroprotective) was studied for these marketed extracts and strong synergistic activity of a mixture was observed

comparing to individual components (Yoshida et al. 2011) underlining beneficial properties of OPCs.

40.1.2 *Theobroma cacao* L.

Theobroma cacao L. is the most widely cultivated among over 20 species in genus Theobroma. It is a source of cacao beans, which were of historical importance in Mesoamerican culture. Cacao drinks were known and popular in Aztec royalty, while beans were used as currency well into the nineteenth century in remote parts of Mesoamerica (Steinberg 2002). Cacao drinks served as a primary remedy or as a vehicle to deliver other medicines. Among over hundred documented traditional uses of cacao, the application for improvement of digestion and elimination, for stimulation of nervous system and induction of weight gain in emaciated patients was the most common (Dillinger et al. 2000). Lower rates of hypertension, cardiovascular disease, obesity, diabetes mellitus, myocardial infarction, stroke, and cancer were observed among native people drinking daily higher amounts of cacao (Katz et al. 2011). The beneficial effects of cacao consumption are related to the action of OPCs (e.g., dimeric procyanidins B1 to B7, trimeric procyanidin C1, tetrameric procyanidin (cinnamtannin A2), and pentameric procyanidin (cinnamtannin A3)) (Esatbeyoglu et al. 2015), which were proved to be more potent than monomeric and polymeric cocoa procyanidins (Dorenkott et al. 2014). The astringent and bitter taste of cacao is also related to the high content of PAs.

40.1.3 *Vitis vinifera* L.

The primary and traditional use of *Vitis vinifera* is wine-making. During this process the pomace containing grape seeds and skin is produced. As being a side product pomace usually was discarded, however, it is a rich source of PAs. Pomace major fraction constitute polymeric procyanidins (PPCs) (65% and 83% in grape seeds and skins, respectively) which are accompanied by lower content of monomeric and OPCs (35% and 17% in grape seeds and skins, respectively) (Luo et al. 2018). The medicinal uses of *V. vinifera* date back to ancient times. European folk treated sore throats with unripe grapes, while dried fruits were used to heal constipation. For such complaints like cholera, smallpox, nausea, eye infections, skin, kidney, and liver diseases, fresh, ripe grapes were used (Badet 2011). Nowadays grape seed extract composes the majority of dietary supplements in the market containing phenolics. It is usually added in the quantity from 50 to 100 mg per capsule, tablet, or granule. Grape seed extract also found application in cosmetics dedicated to skin care and is widely added into different kinds of foods. The pharmacological effects exerted by grape seed extract such as antidiabetic, anti-inflammatory, lowering blood pressure, or reducing plasma cholesterol are related to the presence PPCs and OPCs with B-type linkages (Ashraf et al. 2015; Choy et al. 2013).

40.1.4 *Vaccinium macrocarpon* Aiton

Wild cranberries were discovered by Native Americans, who used its fruits as a food, fabric dye, and a traditional medicine. The primary medical application of cranberries was treatment of bladder and kidney ailments by American Indians (Boon and Smith 2004). Sailors used berries to prevent scurvy. Wounds and blood poisoning were also cured with cranberry fruits poultice (McKay and Blumberg 2007). Till now, cranberries are commercially grown in Canada, the USA, South America, and Europe. The fruits are usually processed before consumption due to low content of sugar and high amount of organic acids and PAs giving berries characteristic tart and astringent taste. Dried cranberries are popular snack and can be found as an ingredient in baked goods, bars, smoothies, trail mixes, cereals, and juice blends. Fresh berries are used for production of juices, jams, and sauces. The folk knowledge about *V. macrocarpon* usefulness in the treatment and prevention of urinary tract infections has been supported by numerous scientific evidence. The bioactive compounds found in cranberry juice are A-type proanthocyanidins, which were proved to inhibit the adhesion of *Escherichia coli* to the epithelium in urinary tract. The direct consequence of the interaction between bacteria and PAs is the inhibition of bacterial biofilm formation (Howell et al. 2005).

40.1.5 *Cinnamomum verum* J.Presl (syn. *Cinnamomum zeylanicum* Blume)

Cinnamomi cortex (the inner bark of Ceylon cinnamon) is known as a spice since the Middle Ages. Earlier, in ancient Egypt, it was used to embalm mummies and to burn in temples and during funerals. In the Middle Ages, cinnamon and other spices were transported by Arabs to Alexandria, Egypt, and then shipped to Europe (Gunawardena et al. 2014). In that time, cinnamon powder was an ingredient of medicines for sore throats and coughs. It was traditionally applied to heal toothaches, dental problems, and bad breath. The known folk application of cinnamon was intestinal spasms, nausea, stomach cramps, indigestion, loss of appetite, or diarrhea (Gunawardena et al. 2014). The effectiveness of cinnamon in prevention and treatment of type 2 diabetes, cancer, and inflammation has recently been shown. Its hypotensive and cholesterol-lowering effects were also promising (Mateos-Martín et al. 2012). Besides its medicinal usefulness, essence and aroma industries use cinnamon in cosmetics and perfumes and in different varieties of foodstuffs due to its characteristic fragrance and flavor. Some activities of cinnamon are attributed to volatile compounds present in the bark; however, majority of studied health benefits was associated with proanthocyanidins, which are main polyphenolic fraction found in commercial cinnamon. Cinnamon PAs are polymers with a degree of polymerization up to 11 and with a high proportion of A-type linkages (Mateos-Martín et al. 2012).

40.1.6 *Sorghum bicolor* (L.) Moench

Sorghum is a drought-tolerant crop, nowadays commonly produced in semi-arid regions of Africa, Asia, Australia, and North and South America. The earliest findings of wild examples of sorghum grains comes from circa 800–600 BCE from Qasr Ibrim in Egyptian Nubia. It was domesticated no earlier than CE 100 (Zohary and Hopf 2000). Traditionally sorghum was grown for grain; however, besides this primary need of food, now sorghum is also used for forage and sugar (sweet stalk) production. The bioactive compounds (polyphenols and lipids) are mainly located in the bran fraction of sorghum grains. These compounds are accompanied with the abundant cell wall polysaccharides and contribute significantly to the health benefits attributed to whole grain intake (Girard and Awika 2018). Consumption of sorghum grains is related to reduction of oxidative stress and chronic inflammation, prevention of cancer, improvement of glucose metabolism, prevention of insulin resistance, and improvement of lipid metabolism (Girard and Awika 2018). The PAs fraction in sorghum constitutes up to 5%, what is tenfold higher than in other grains. It is composed of catechin/epicatechin oligomers and polymers with the degree of polymerization up to 20 (Girard and Awika 2018).

40.2 Bioactive Constituents

PAs present a wide structure variability which is determined by stereochemistry at the chiral centers and the hydroxylation pattern of flavan-3-ol units. 2*R*-type flavan-3-ols are predominantly produced by plants whereas S*R*-type flavan-3-ols can rarely be found in monocotyledons and in selected dicotyledonous families like *Rhus*, *Uncaria*, *Polygonum*, *Raphiolepis*, and *Schinopsis* (He et al. 2008). The location and stereochemistry of the interflavan linkage between extension and end units as well as degree of polymerization (DP) also influences the complexity of final oligomeric and polymeric structures (He et al. 2008). Two main types of linkages between monomer units can be observed: A-type, when C2 position of the upper unit is linked to the oxygen at C7 or C5 position of the lower unit and C4 position of the upper unit is linked to the C8 or C6 of the lower unit (Fig. 1), and B-type, when C4 position of the upper unit is linked to the C8 or C6 of the lower unit (Fig. 2). Mixed-type PAs combine both kinds of linkages, which can be either α or β (Fig. 3) (He et al. 2008). The clear nomenclature of PAs includes corresponding flavan-3-ol monomers and indicates the configuration (described as α or β), location, and direction of interflavan linkage using parentheses with an arrow (→). The examples of names of oligomers are presented on Figs. 1, 2, and 3.

The chemical term proanthocyanidins suggests they can produce anthocyanins. Indeed, catechin- and epicatechin-based oligomers or polymers are known as procyanidins because upon acid hydrolysis they yield anthocyanin aglycon cyanidin. Similarly, gallocatechin- and epigallocatechin-based polymers (prodelphinidins) are the source of anthocyanin aglycon delphinidin. The other subgroups of PAs classified according to the hydroxylation pattern are presented in the Table 1. Among these

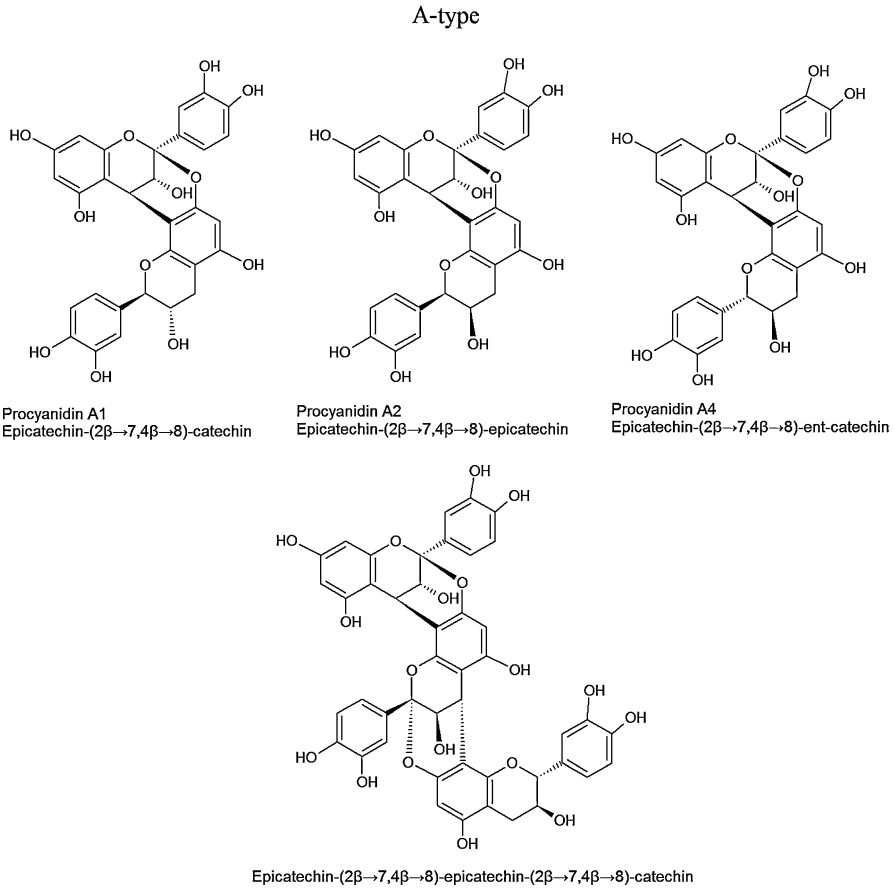


Fig. 1 The structures of A-type proanthocyanidins

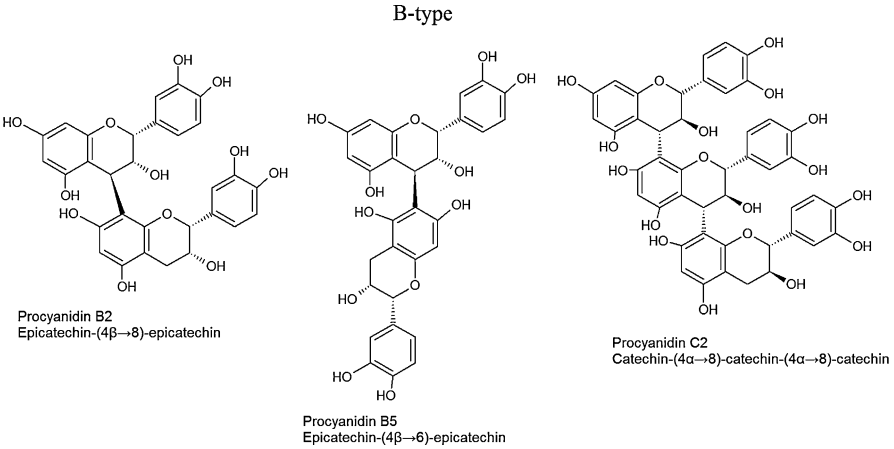


Fig. 2 The structures of B-type proanthocyanidins

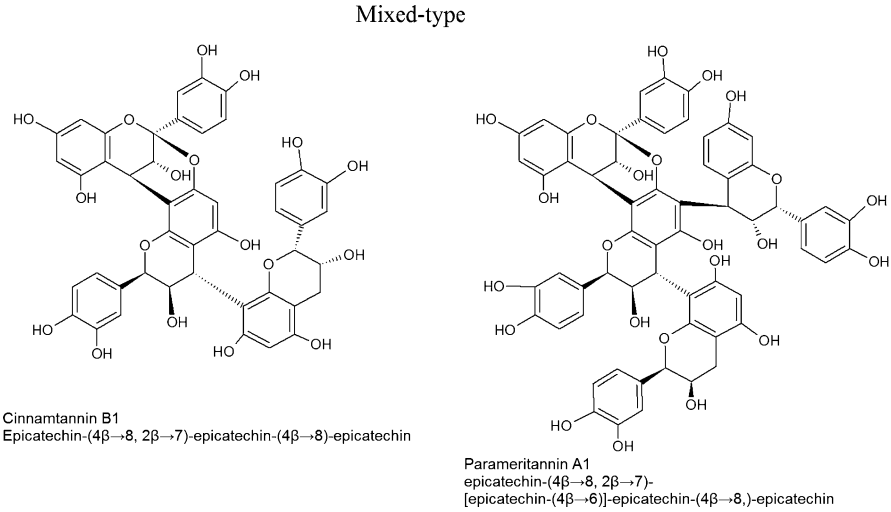
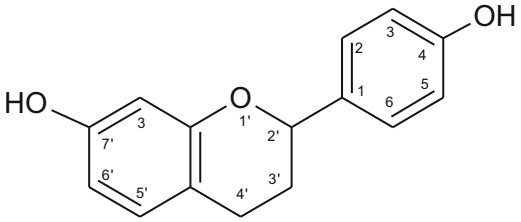


Fig. 3 The structures of mixed-type proanthocyanidins

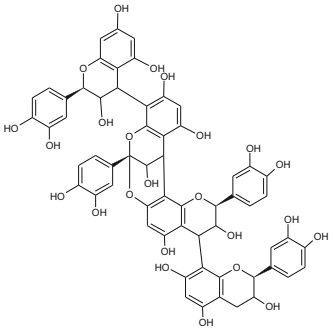
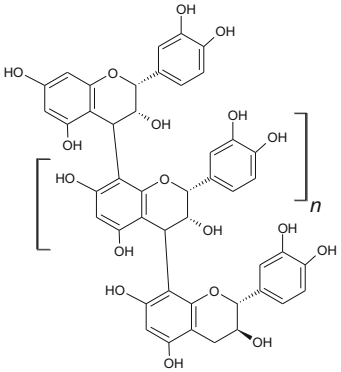
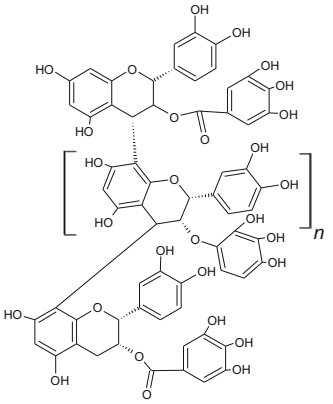
Table 1 The hydroxylation pattern of proanthocyanidins



	Carbon number				
PAs subgroup	3	5	3′	5′	8′
Procassinidin	H	H	H	H	H
Probutinidin	OH	H	H	H	H
Proapigeninidin	H	H	H	OH	H
Proluteolinidin	OH	H	H	OH	H
Protricetinidin	OH	OH	H	OH	H
Propelargonidin	H	H	OH	OH	H
Procyanidin	OH	H	OH	OH	H
Prodelphinidin	OH	OH	OH	OH	H
Proguibourtinidin	OH	H	H	H	H
Profisetinidin	OH	H	OH	H	H
Prorobinetinidin	OH	OH	OH	H	H
Proteracacinidin[a]	H	H	OH	H	OH
Promelacacinidin	OH	H	OH	H	OH

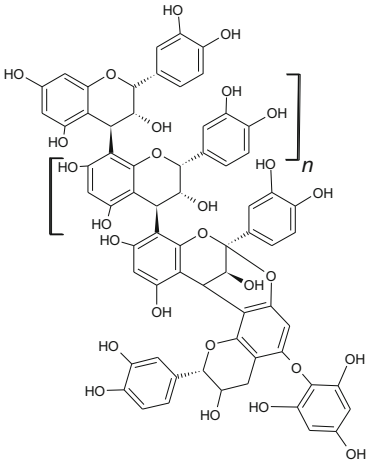
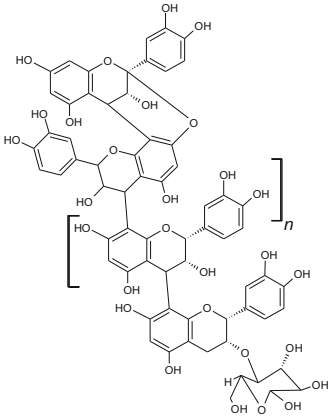
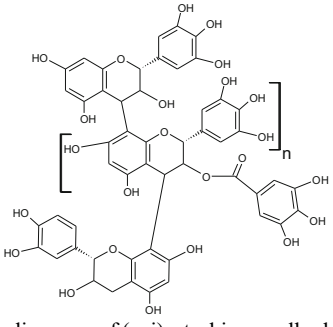
[a]only synthetical

Table 2 Bioactive constituents of plants rich in proanthocyanidins

Plant source	PAs	Structure	References
Cinnamon bark	Combinations of (epi)catechin, (epi) catechin gallate, (epi) gallocatechin, and (epi)afzelechin, resulting in a highly heterogeneous mixture of procyanidins, prodelphinidins, and propelargonidins of DP up to 11	 (epi)catechin tetramer	Mateos-Martín et al. 2012
Sumac grain	B-type (epi)catechin oligomers and polimers	 (epi)catechin oligomers and polimers	Wika et al. 2003
Grape seed	Procyanidin dimers B1, B2, B3, B4, B5, B7, B6, and B8, procyanidin trimers, galloylated oligomers of DP up to 14	 galloylated (epi)catechin oligomers and polymers	Liu and White 2012

(continued)

Table 2 (continued)

Plant source	PAs	Structure	References
Cranberry fruits	Dimers, trimers and oligomers of epicatechin with phloroglucinol adducts	 oligomers of epicatechin with phloroglucinol adducts	Foo et al. 2000
Raw cacao beans	Procyanidin B-type oligomers (DP4–DP11) and oligomers of procyanidin A-type glycosides	 oligomers of procyanidin A-type glycosides	D'Souzaa et al. 2017
Pinus bark, Pycnogenol®	Procyanidin B-type oligomers of (epi) catechin or galloylated (epi) catechin of DP ranging from five to seven	 oligomers of (epi)catechin or galloylated (epi)catechin	Kim et al. 2012

subgroups, procyanidins are the most common one. Mixed polymers composed of several types of units can also be found in nature (He et al. 2008; Sieniawska and Baj 2016; Wallace 2010). The main bioactive constituents of plants rich in PAs are presented in the Table 2.

40.3 Bioavailability and Metabolism

Although the beneficial effects have been observed as a result of dietary PAs intake, a more in-depth knowledge of their metabolic pathways in human and animals is still necessary. The low absorption rate of PAs is well-known and can lead to favorable direct activity in the gastrointestinal tract. PAs are found in both forms of oligomers and polymers, but polymers are the most abundant in foods. While oligomers are absorbable *in vivo*, the high molecular weight of the polymers determines their poor absorbability in the gastrointestinal tract.

After ingestion procyanidins undergo metabolic modifications in the gastrointestinal tract. These modifications take place from mouth to colon and result in smaller oligomeric molecules or metabolites with higher bioavailability and thus higher *in vivo* bioactivity.

40.3.1 Influence of Saliva and Gastric Juice

It is known that the first organ involved in the modification and digestion of PAs is the small intestine. However, the role of the two fluids encountered before reaching the small intestine (saliva and gastric juice) must be investigated.

Even though PAs are not metabolized in the oral cavity, however, studies on the role of saliva in altering their structure have been conducted (Zanotti et al. 2015). PAs interactions with salivary proteins were investigated in a study using synthesized procyanidin dimers, C1 trimer, (−)-epicatechin O-gallate, and B2-3″-O-gallate from grape seeds. The results showed a higher tannin-specific affinity to saliva proteins of (+)-catechin, as well as of procyanidin dimers linked through a C4–C8 interflavan bond (de Freitas and Mateus 2001).

In a study conducted on green tea extracts of 5.0 mg/ml administrated as mouth rinse solution for caries prevention, it was found that, eight catechins were retained in saliva at μg/ml levels after 1 h (Tsuchiya et al. 1997).

Because the pH of the gastric juice generally varies between 1 and 3, it is necessary to know the behavior and stability of PAs in such an environment. Two hypotheses may be taken into account: acidic conditions lead to the degradation of PAs to monomers, and as a result, they would not get intact in the small intestine, or PAs are stable under acidic conditions, and in this case, they can be absorbed into the small intestine and detected in the plasma (Zhang et al. 2016). *In vitro* studies have been initiated to simulate the conditions encountered in the stomach, thus constituting a good indicator of the potential bioavailability of procyanidins *in vivo* (Spencer et al. 2001). The results showed that at pH ranging from 1.8 to 2, (−)-epicatechin

was stable, while B2 dimer and procyanidins oligomers degraded into (−)-epicatechin, and dimers and monomers, respectively; on the contrary, while procyanidins oligomers were stable at alkaline pH, (−)-epicatechin and B2 dimer were unstable, and at nearly neutral pH, the latter was stable (Zhu et al. 2002; Kahle et al. 2011; Spencer et al. 2000; Zhang et al. 2016).

The results obtained in *in vitro* studies have not been completely confirmed *in vivo*. On the one hand, the number of such studies is still very small, and on the other hand, besides pH influence, the food matrix may play an important role in PAs digestion as well. In a study conducted by Rios et al. (2002), six human subjects consumed 500 ml of cocoa beverage containing 733 mg of OPCs. At pH of 6.5 monomers, dimers B2 and B5, C1 trimer, as well as oligomers had similar HPLC profiles in the gastric content extracts and in the cocoa beverage indicating that most of the ingested procyanidins reached the small intestine as intact molecules. This could be explained by the higher pH value (5.4) determined by the food bolus.

40.3.2 Metabolism in Small Intestine and Colon

The pathways of PAs absorption can be through the stomach or intestine. However if not absorbed at this level, PAs reach colon and undergo the modification by the microbiota. During this process, PAs come under catabolic or conjugation reactions, then pass into blood, and are subsequently eliminated either through the urinary bladder or through the bile; some unabsorbed ones are eliminated through feces.

Although the small intestine is considered the first organ involved in PAs digestion, there is no evidence of mammalian enzymes that can degrade this type of high molecular weight molecules. It has been estimated that only 5–10% of the total ingested polyphenols are absorbed in the small intestine. The rest is accumulated almost intact in the large intestine (Zanotti et al. 2015). In fact, only procyanidin B2 was found to be absorbed intact. In a study investigating the possibility of quantifying the plasma levels of procyanidins after consumption of a flavanol-rich cocoa, Holt et al. (2002) detected procyanidin dimer in the plasma of human subjects after 0.5 h. The concentration reached a maximum at 2 h after consumption (16 ± 5 nmol/L, 41 ± 4 nmol/L, respectively). The other oligomers, with higher polymerization degree, reached colon and were metabolized by the colonic microbiota (Williamson and Clifford 2017).

Because PAs are absorbed in the small intestine in small proportions, a massive accumulation of several hundred micromoles/l is obtained in the colon (Choy and Waterhouse 2014). The behavior of procyanidins oligomers and polymers in the colon is not fully elucidated, and the results of the studies are sometimes contradictory. However, it has been demonstrated that metabolites with lower molecular weight, such as phenyl valerolactone, phenylacetic acids, and phenylpropionic acids, result after procyanidins are catabolized by colonic microflora (Zhang et al. 2016). The potential biological effects of procyanidins are attributed to these gut

Table 3 Metabolites produced by colonic bacteria

PA source	Compound	Metabolites	References
Grape seed	PAs	Hydroxyphenyl acetic acid, Hydroxyphenyl valeric acid	Zhang et al. 2016
Litchi chinensis	PAs dimer A2 Cinnamtannin B1	3,4-Dihydroxyphenylacetic acid and 3-hydroxyphenylpropionic acid, 4-hydroxyphenylacetic acid, 3-hydroxyphenylpropionic acid, and 3,4-dihydroxyphenylacetic acid.	Zhang et al. 2016
Cocoa	(−)-Epicatechin	(−)-Epicatechin-3′-O-glucuronide	Zanotti et al. 2015
Tea	(−)-Epigallocatechin-3-O-gallate	–	Zanotti et al. 2015
Cocoa	Dimers and other procyanidin oligomers	Epicatechin monomers	Holt et al. 2002
Green tea	Monomeric or dimeric flavan3-ols	Glucuronide, hydroxybenzoic acid, hydroxyphenylacetic acid, hydroxyphenylpropinoic acid, hyrdroxyphenylvaleric acid, hydroxycinnamic acids	Choy and Waterhouse 2014

microbiota metabolites, rather than parent compounds. The metabolites described are presented in Table 3.

In a study conducted on rats by Baba et al. (2001), urine absorption and excretion of (−)-epicatechin was monitored. After administration of both, cocoa powder (in different doses) and equivalent doses of the (−)-epicatechin, the sum of metabolites of (−)-epicatechin in plasma and urine, excreted after 18 h postadministration, was measured. The results showed a linear dose increase and, moreover, an equal level of the amount of (−) epicatechin metabolites in the urine for both types of administration.

Opposite results have been obtained by Donovan et al. (2002), in a study in which the rats were fed with catechins, procyanidin B3 dimer, and grape seed extract containing catechin, epicatechin, and a procyanidin mixture at a single meal. The results showed the presence of catechin and epicatechin conjugated forms, but the absence of procyanidins or their conjugates in plasma and urine. Also, the mixture present in the grape seed extract showed no bioavailable monomers or any effects on plasma levels or urinary excretion.

The microbial metabolism of two A-type procyanidins, procyanidin A2 and cinnamtannin B1, was studied by incubation in a pig cecum model. It was found that both A-type procyanidins were degraded by the microbiota; the procyanidin A2 was degraded by about 80%, while cinnamtannin B1 about 40% after 8 h of incubation. The main identified metabolites were 3,4-dihydroxyphenylacetic acid and 3-hydroxyphenylpropionic acid and 4-hydroxyphenylacetic acid, 3- hydroxyphenylpropionic acid,

and 3,4-dihydroxyphenylacetic acid, respectively (Zhang et al. 2016; Engemann et al. 2012).

In a comparative study conducted *in vitro* and *in vivo*, Serra et al. (2011) investigated colonic metabolism for catechin, epicatechin, B2 dimer, epicatechin gallate (EGC), and epigallocatechin gallate (EGCG) by incubating each individual standard with rat fecal suspension for 48 h, by fermenting a cocoa cream with high-procyanidins content, previously digested by *in vitro* digestion, and also by *in vivo* analysis of rat large intestine and intestinal content after a single intake of the same nuts – cocoa cream. The results allowed the assumption of the existence of two metabolic pathways – dehydroxylation and rupture of the 1–2 bond of the C ring – considering the presence of diarylpropan-2-ol and 5-(3,4-dihydroxyphenyl)-c-valerolactone in the catechin and epicatechin fermentation mediums. The metabolism of ECG and EGCG did not yield the equivalent valerolactone with three hydroxylations, and the metabolites of dimer B2 differed from the ones of epicatechin, phenylacetic, and 4-hydroxyphenylacetic acids being the only common metabolites. *In vivo* verification of the metabolic pathways led to the quantification of phenylacetic acid, 3-hydroxyphenylacetic acid, and 4-hydroxyphenylacetic acid as main metabolites and of two valerolactones 5-(hydroxyphenyl)-c-valerolactone and 5-(3,4-dihydroxyphenyl)-c-valerolactone in lower levels.

Several *in vivo* studies were conducted in order to detect the microbial-derived metabolites in urine, after intake of procyanidins-rich diets. As a result, an increase in 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenyl-γ-valerolactone was found in rats' urine after being fed with procyanidin B3, but neither parent compound nor catechin derivatives could be detected (Choy and Waterhouse 2014; Gonthier et al. 2003). In case of procyanidin trimer C2 intake, the main metabolites found in urine were 3- hydroxyphenylvaleric, 3-hydroxyphenylpropionic acid, and m- coumaric acids (Gonthier et al. 2003). In another study, the absorption of procyanidins was investigated, after apple peel extract was administrated to rats catechin, epicatechin, procyanidin B1 and B2, and procyanidin C1 were detected in rat's plasma, with a maximum level registered at 2 h after administration, and decreasing after until 24 h (Shoji et al. 2006).

In a study investigating the structurally related –(−) epicatechin metabolites present in human systemic circulation, after the consumption of a cocoa dairy-based drink containing –(−) epicatechin, Ottaviani et al. (2012) identified –(−)epicatechin –glucuronide, –(−)epicatechin sulfates as the main metabolites in humans, reaching a maximal plasma levels at 2 h after consumption of the test drink (589 ± 85 nM, and 331 ± 26 nM, respectively). Also unmetabolized –(−)epicatechin was detected in circulation but at low concentration of about 4 nM at 1 h after drink consumption.

Having complicated structures and high molecular weight, the absorption of procyanidins in the small intestine is very ineffective, and therefore the colon remains the main organ responsible for the bioavailability of the dietary procyanidins.

40.4 Bioactivities (Animal Aspects)

40.4.1 Extracts Rich in Procyanidins

The activity of procyanidins present in such plants as cocoa, grape, maritime bark, etc. is intensively studied in animal models (usually rats). Researches in this field are mainly focused on study of diabetes, coronary, or gastrointestinal diseases. Some other effects of procyanidins on other affections are less studied.

The activity of extracts and the main isolated compounds discussed in this chapter is presented in Table 4.

Table 4 Extracts and plants sources with the main compounds

Plant source/ extract	Compounds	Activity	References
Commercially available Pycnogenol	Mixture of procyanidins	Antidiabetic	Maritim et al. 2003
Diospyros kaki peel	Mixture of procyanidins	Antidiabetic	Lee et al. 2007
Grape seeds	Monomeric, dimeric, trimeric, tetrameric, oligomeric procyanidins	Antidiabetic	Montagut et al. 2009
Provinols™	–	Antidiabetic	Agouni et al. 2009
Grape seeds	Proanthocyanidins	Antidiabetic	Li et al. 2009
Cacao liquor	Procyanidin B2 Procyanidin C1 Cinnamtannin A2 Galactopyranosyl-ent-(2)-epicatechin-(2)-epicatechin	Anticancer	Yamagishi et al. 2002
Grape seeds	Procyanidin B1 procyanidin B2 procyanidin B3 procyanidin B4 procyanidin B5	Anticancer	Mittal et al. 2003
Ginkgo extract	Proanthocyanidins	Neurological diseases	Cao et al. 2016
Grape seeds	PAs	Metabolic syndrome	Ibars et al. (2017)
Cacao	PAs	Metabolic syndrome	Osakabe and Yamagishi (2009)
	Flavan-3-ols procyanidin dimers	Cardiovascular and metabolic disorders	Leonetti et al. (2018)
Grape seeds	Proanthocyanidins	Antithrombotic effect	Zhang et al. (2011)
Grape seeds	Procyanidin dimer, tetramer, pentamer, heptamer oligomers	Anticataract activity	Yamakoshi et al. (2002b)

40.4.1.1 Antidiabetic Activity

Pinent et al. (2012), in their review article *Procyanidins Improve some Disrupted Glucose Homoeostatic Situations: An Analysis of Doses and Treatments According to Different Animal Model*, analyzed the potential beneficial effects of PAs, in situations in which glucose homeostasis is disrupted. They observed that several authors have assayed the ability of procyanidin-enriched extracts to ameliorate the physiological state caused by inability to synthesize and/or secrete functional insulin (hyperglycemia in type 1 diabetes). Maritim et al. (2003) used the commercially available Pycnogenol product (at a dose of 10 mg/kg) for the treatment of streptozotocin-induced diabetes in female Sprague Dawley rats (14 days treatment). A decrease of serum glucose level at day 15 was observed. At the same time the safety of tested product was confirmed on normal or diabetic rats. The results obtained by the authors (elevated levels of reduced glutathione and glutathione redox enzyme activities) demonstrated the ability of Pycnogenol (standardized extract of *Pinus maritima* consisting of a mixture of procyanidins) to scavenge free radicals altered oxidative stress and to increase the activity of hepatic-glutamyl-transpeptidase, which normally hydrolyzes glutathione and thiol derivatives and conserves cysteine levels.

Lee et al. (2007) evaluated the antidiabetic and antioxidant potential of PAs obtained from persimmon peel on male Wistar STZ-diabetic rats. At 24 h from the last dose, the decrease in serum glucose and glycosylated protein was observed.

The probable mechanisms involved in beneficial effects of PAs in diabetes mellitus are limitation of post-prandial glycemia increases, inhibition of α-glucosidases and α-amylases, and inhibition of transporters involved in glucose uptake (e.g., glucose transporter type 2 – Glut2) (Kwon et al. 2007).

In long-term treatments, procyanidins which are administered in a daily treatment are more effective than when given with food, which indicates the presence of mechanisms of action other than those inhibiting carbohydrate digestions and/or glucose absorption. Thus these compounds are acting as an insulin-mimetics (Pinent et al. 2012).

Other authors suggest that procyanidins have insulin-mimetic effects in adipose tissue and muscle. Montagut et al. (2009) proved that oligomeric PAs of a grape seed extract interact and induce the autophosphorylation of the insulin receptor in order to stimulate the uptake of glucose, however, in different manner than insulin. It was found that Akt (protein kinase B) and MAPK proteins are essential for PAs signaling mechanisms. The ability of PAs to mimic insulin effects in insulin sensitive targets was demonstrated also in healthy animal models when insulin is scarce or absent. Wistar rats and Zucker lean rats used as healthy model simultaneously with diabetic animals demonstrated no changes in glucose level after 8 and 24 weeks of treatment, respectively (Agouni et al. 2009; Li et al. 2009).

40.4.1.2 Anticancer Activity

Yamagishi et al. (2002) observed that cacao liquor procyanidins inhibit *in vitro* mutagenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and rat pancreatic carcinogenesis in the initiation stage. However such promising results were not

confirmed in rat mammary carcinogenesis induced by PhIP model. The same authors evaluated also chemopreventive properties of cacao liquor proanthocyanidins against lung carcinogenesis in F344 male rat multi-organ carcinogenesis model. PAs were effective as chemopreventive agents in the lung cancer; however, none protecting influence in other major organs was observed (Yamagishi et al. 2003).

Mittal et al. (2003) studied how grape seeds procyanidins can prevent UVB radiation-induced photocarcinogenesis and malignant conversion of benign papilloma to carcinomas in SKH-1 hairless mouse model. They introduced PAs into the diet of hairless mice in order to demonstrate the effect of the compounds on different stages of photocarcinogenesis, such as UVB-induced tumor initiation stage, UVB-induced tumor promotion stage and UVB-induced complete carcinogenesis. The used composition was based on dimers (containing procyanidin B1, procyanidin B2, procyanidin B3, procyanidin B4, and procyanidin B5) - 6.6% of total grape seed procyanidins, trimers (containing procyanidin B5-3′-gallate and procyanidin C1) – 5.0%, tetramers – 2.9%, and oligomers – 74.8%. The results presented by the research group showed that this diet prevented photocarcinogenesis, in terms of tumor incidence, tumor multiplicity, and tumor size. PAs showed also protective action against UVB-induced skin tumorigenesis in all the three stages of multi-stage carcinogenesis. These compounds have inhibitory effect on malignant conversion of benign skin papilloma to carcinomas in SKH-1 hairless mice. The observed positive effects were explained by probable antioxidant activity of PAs (Mittal et al. 2003).

40.4.1.3 Neurological Diseases

The administration of procyanidins in neurological diseases was also studied. PAs were showed to effectively protect ischemic neurons, but the mechanism remains poorly understood. The intraperitoneal administration of ginkgo proanthocyanidins in Sprague Dawley rats mitigated neurological disorders, shortened infarct volume, increased superoxide dismutase activity, and decreased malondialdehyde and nitric oxide contents. PAs inhibited inflammatory reaction and activated survival pathways after ischemia/reperfusion injury (Cao et al. 2016).

40.4.1.4 Metabolic Syndrome

Ibars et al. (2017) studied the effects of procyanidins from grape seeds on rats with diet-induced obesity. Dietary obesity is usually linked with hypothalamic leptin resistance, and the authors presented the role of proanthocyanidins on hypothalamic leptin/STAT3 (signal transducer and activator of transcription 3) signaling and pro-opiomelanocortin gene expression on male Wistar rats fed either a standard chow diet or a cafeteria diet for 13 weeks, followed by treatment with grape seed proanthocyanidin extract. The treatment activated the hypothalamic leptin receptor-STAT3 pathway, ameliorated food intake but did not reverse the obesity and hyperleptinemia. Rats treated with procyanidins did not display a significant body weight reduction indicating that the doses were not sufficient to totally reverse leptin dysfunction induced by a high-fat diet.

Osakabe and Yamagishi (2009) investigated cacao procyanidins (CP) effects on plasma lipid levels in high cholesterol-fed rats. The experiments were developed on

9-week-old male Sprague Dawley rats. They demonstrated that ingestion of cocoa procyanidins reduced plasma total cholesterol levels in rats fed with high cholesterol diet, and the accumulation of cholesterol and triglyceride in liver was significantly decreased. The PAs precipitated micellar cholesterol, and this ability was dependent on the molecular weight of the compounds (monomers to tetramers).

The effect of an extract enriched in the flavan-3-ols procyanidin dimmers on obesity-related disorders via estrogen receptor alpha (ERα) was investigated by Leonetti et al. (2018). The investigated obesity-related cardiovascular and metabolic disorders with a particular interest in the role/contribution of ERα revealed that procyanidins reduced adiposity, plasma triglycerides, and oxidative stress in the heart, liver, adipose, and skeletal tissues, but did not improve the vascular dysfunction in a 2-week treatment. The heart structure and function were not affected by the diet, nor the grape seed extract supplementation and reactive oxygen species production in aorta was not significantly modified. The treatment improved muscle function by activating β-oxidation and by increasing mitochondrial functionality. ERα was identified as an important target involved in the reduction of fat accumulation.

40.4.1.5 Antithrombotic Activity

Zhang et al. (2011) studied antithrombotic effect of grape seed proanthocyanidins. Deep vein thrombosis was induced in rats, and a recipe based on 400 mg/kg procyanidins was applied. The authors observed that active compounds reduced thrombus length and weight and protected the integrity of the endothelium, inhibited thrombogenesis-promoting factors, and promoted thrombogenesis-demoting factors CD34, vascular endothelial growth factor receptor-2, and ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type one motif, member 13). The antithrombotic properties of proanthocyanidins were associated with endothelial protection and regeneration, platelet aggregation, and inhibition of inflammatory cell and thrombus adhesion.

40.4.1.6 Other Activities

Schizophrenia is mental disorder with disturbances in emotion, perception, cognition, and social function. Although this illness affects 1% of the population worldwide, fundamental neuropathology of schizophrenia remains unexplained. Tian et al. (2018) demonstrated how procyanidin B2 protects myelin integrity in cuprizone-induced schizophrenia in mice. They evaluated the effects of procyanidin B2 widely available in food on behavioral impairment in mice exposed to cuprizone for 5 weeks. Behavioral impairment tests, myelin integrity assay, and myelin basic protein (MBP) expression revealed that procyanidin B2 could mitigate behavioral impairment and protect myelin integrity via regulating oxidative stress by activating nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling.

Yamakoshi et al. (2002b) studied the effect of PAs from grape seeds on cataract formation on a 39 male ICR/f group of rats. The diet was based on mixture of procyanidin oligomers (DP between 2 and 10) but also polymers in total concentration of 38.5% and 2.4% monomeric flavanols such as (+)-catechin. PAs were found

to significantly prevent and postpone the development of cataract formation in rats. The comparison of effectiveness of procyanidin structures with different degrees of polymerization resulted in conclusion that procyanidin pentamer to heptamer and procyanidin oligomers more than decamer groups were significantly more efficient in lowering plasma cholesteryl ester hydroperoxide levels comparing to the procyanidin dimer to tetramer group. The antioxidant properties of PAs and their metabolites were suggested to contribute to the inhibition of progression of cataract formation.

40.5 Benefits (Human Studies)

The 67 clinical trials involving PAs can be found in *Cochrane Database of Systematic Reviews.* Some of them are exemplified in the next subchapters, being classified according to the treated diseases.

Additionally, a summary of clinical trials investigating procyanidins and meta-analysis of these is presented in Table 5. An important source of PAs used in such cases are fruits and berries (blueberries, cranberries, and black currant), some legumes (peas and beans), hazelnuts, pistachios, almonds, walnuts and cocoa, and spices as cinnamon. From the fruits we can mention apples, chokeberries, strawberries, and green and red grapes (especially grape seeds) and the product obtained from grapes: the wine.

40.5.1 Cancer

As a disease of the century with a particular severity, cancer is considered the second leading cause of death worldwide. The major concerns of the scientific and medical world are to find cures for this disease, due to the fact that conventional cancer therapies result in serious side effects and, at best, merely extend the patient's life span by a few years. Formulations based on natural products can be considered good alternatives to traditional treatments.

Procyanidins obtained from grapes and grape seeds are considered good candidates for the anticancer formulations. Santos-Buelga and González-Manzano (2011) considered that excessive alcohol consumption increases the risk of liver cirrhosis and cancers; however, low to moderate red wine consumption has been associated to health-promoting properties. Among the wine phenolics, the authors assign the claimed health effects to procyanidins (such as oligo- and polymeric flavan-3-ols) and to resveratrol.

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world, with a high percent of incidence among all types of cancers. Jin et al. (2012) have studied the effect of dietary flavonoids on the incidence of colorectal adenoma and CRC. At the start of Jin's study, only few researchers investigated the association between procyanidins and colorectal neoplasms, while others reported the effects of six flavonoid subclasses (flavonols, flavones, flavan-3-ols,

Table 5 Summary of clinical trials investigating procyanidins

Compound	Clinical trial/ meta-analysis	Effects	Reference
PAs	Analysis of metadata	Decreasing risk of colorectal cancer (CRC)	Theodoratou et al. 2007
PAs from commercially available standardized cranberry capsules	Clinical trial	Potential to attenuate the adverse effects of cancer radiotherapy	Hamilton et al. 2015
Dietary biologic active compounds from wine, apples, pears, peaches, apricots, prunes, vegetables/ bean soups, chocolate, pulses, and grapes	Analysis of metadata	Reduce endometrial cancer risk	Rossi et al. 2013
Cocoa procyanidins	Clinical trial	Increase of flow-mediated vasodilation Decrease of the pulse wave velocity and blood pressure Decrease of total cholesterol	Heiss and Kelm 2016
Cocoa procyanidins: procyanidin dimers and trimers to decamers	Clinical trial	Improving cardiac function in chronic heart failure, changing blood pressure, decreasing flow-mediated dilatation	De Palma et al. 2016
Apple procyanidins	Clinical trial	Changes in systolic or diastolic BP (peripheral and aortic), plasma nitric oxide (NO) reaction products	Kroon 2016
Enovita®, grape seeds	Clinical trial	Decrease of blood pressure, decrease of the diastolic pressure, oxidation of membrane lipids, endothelial dysfunction, formation of oxidized LDL, and activation of phagocytic cells	Belcaro et al. 2013
Oligopin®	Clinical trial	Improve lipid cardiovascular profile and one of the scarce ways to increase HDL-c in stage-1-hypertensive subjects, improve systolic BP and LDL oxidation	Valls et al. 2016
PAs from cinnamon	Clinical trial	Moderate decrease of fasting plasma glucose, but no effect on glycated hemoglobin A1c, serum lipids, or blood coagulation parameters	Mang et al. 2006
Cinnamon	Clinical trial	Significant reduction in blood pressure, fasting plasma glucose, body mass index	Akilen et al. 2010

(continued)

Table 5 (continued)

Compound	Clinical trial/ meta-analysis	Effects	Reference
PAs from cinnamon	Clinical trial	No significant reduction in glucose values; No changes in the blood lipid profile	Vanschoonbeek et al. 2006
PAs from cinnamon	Clinical trial	Significant decrease of fasting insulin, glucose, total cholesterol and LDL cholesterol The increase of insulin sensitivity	Anderson et al. 2015
Cranberry-lingonberry juice	Clinical trial	Reduction of urinary infection risk	Kontiokari et al. 2001
Cranberry juice capsules	Clinical trial	Preventing urinary tract infections (UTI) after surgery	Foxman et al. 2015
Cranberry juice	Clinical trial	Reduction of bacteriuria incidence (especially *E. coli*) in urinary infections	Wan et al. 2016
Oligomeric and polymeric proanthocyanidins from *Rumex acetosa* L.	Clinical trial	Prophylactic potential on periodontitis	Beikler 2014
Pycnogenol®	One-patient study	Decrease the hyperactivity and impulsivity (in attention deficit hyperactivity disorder (ADHD))	Heimann 1999
Pycnogenol®	Clinical trial	Significant reduction of hyperactivity, improvement of attention and visual-motoric coordination and concentration of children with ADHD	Trebaticka et al. 2006

procyanidins, flavanones, and phytoestrogens) (Theodoratou et al. 2007). Theodoratou's results showed a statistically significant decreased risk of CRC with a high intake of procyanidins (22% reduction in CRC risk associated with the highest quartiles of intake versus the lowest quartile, odds ratio 0.78, $P_{trend} = 0.031$). Their research method was based on the analysis of metadata up to July 2011 in the Cochrane Library, PubMed, EMBASE, and other CINAHL databases and reference lists of previous reviews. Eight studies with 390,769 participants were included. Five studies used a prospective cohort design, two case-control studies, and one a randomized controlled trial (RCT). The methodological quality was measured using the Newcastle Ottawa scale (NOS).

In the clinical trial developed by Hamilton et al. (2015), PAs were evaluated for their potential to attenuate the adverse effects of cancer radiotherapy. The authors used commercially available standardized cranberry capsules, containing 72 mg of

proanthocyanidins, for the evaluation of their effect in prevention and treatment of radiation cystitis in prostate cancer patients. The authors reported decrease in cystitis incidence in treated patients compared with control group, recommending the use of cranberry capsules as a support treatment in prostate cancer patients experiencing common side effects, such as radiation cystitis and inflammation of the bladder.

Rossi et al. (2013) examined the relation between dietary biologic active compounds and endometrial cancer, from an Italian case-control study including 454 incidents, histologically confirmed endometrial cancers, and 908 hospital-based controls. They estimated the intake in proanthocyanidins from fruits and vegetables through statistical tests and concluded that high consumption of proanthocyanidins may reduce endometrial cancer risk. In their study, the authors identified, for the Italian population, as major source of proanthocyanidins monomers and dimers the wine, apples, pears, peaches, apricots, and prunes while for trimers and superior proanthocyanidins the apples, pears, wine, vegetables/bean soups, chocolate, pulses, and grapes.

40.5.2 Cardiovascular Disorders

The use of biologic compounds from plants and fruits offers potential benefices in human health. In the literature there are many papers which describe the role of dietary food, also clinical trials which prove an improvement of health. In the comprehensive review paper, Schroeter et al. (2010) listed several studies on the potential health benefits of dietary flavanols and procyanidins, especially in the context of cardiovascular health. The work was revisited 8 years later (Ottaviani et al. 2018) by authors describing significant developments in understanding of dietary flavanols and procyanidins in the human health and nutrition. In spite of these findings, the recent progress was considered by the authors insufficient for the development of dietary guidance on the flavanols and procyanidins intake or for the establishment of a minimum intake necessary to achieve health benefits.

The effects of cocoa procyanidins on vascular function were investigated in the trial NCT02728466 (Heiss and Kelm 2016). The study started in 2014 involved group of 45 participants. The tests were performed with cocoa-based supplement containing flavanols (monomers) and procyanidins (dimers to decamers) and a placebo comparator (flavanol and procyanidin deprived supplement). The study evaluated the influence of the sustained intake (2x daily over 1 month) of the macro- and micronutrient matched supplement over the baseline endothelial function. Post-consumption, endothelial function, plasma flavanol metabolites, urinary flavanol metabolites, urinary valerolactone metabolites, pulse wave velocity, blood pressure, and high- and low-density lipoproteins were measured at different times. The obtained results showed that the consumption of cocoa extract containing 130 mg (−)-epicatechin and 560 mg procyanidins led to the increase of flow-mediated vasodilation and of the concentration of (−)-epicatechin metabolites in the circulatory system and to a decrease of the pulse wave velocity and blood

pressure. The total cholesterol decreased for both the above described recipe and for another extract (containing 20 mg (−)-epicatechin and 540 mg procyanidins).

De Palma et al. (2016) assessed the potential therapeutic value of a high dose of cocoa flavanols in patients with chronic heart failure. The reductions in N-terminal pro-B-type natriuretic peptide (NT-proBNP) were used as an index of improved cardiac function. The study was a single-center randomized double-blind placebo-controlled investigation with a crossover design with exclusion criteria: age <45 years, diabetes mellitus; LV (left ventricular) dysfunction not related to systolic HF (heart failure) or ischemic heart disease; exertional angina; atrial fibrillation; cardiac surgery, percutaneous coronary intervention, acute coronary syndrome, or stroke within 6 months of the study; active psychiatric or psychological illness; and life expectancy. The patients' diet was rich in flavanols, especially procyanidin dimers and trimers to decamers. This study indicates that combining cocoa flavanols with guideline-directed medical therapy has potential for improving cardiac function in chronic heart failure, changing blood pressure, decreasing flow-mediated dilatation, using reductions in N-terminal pro-B-type natriuretic peptide (NT-proBNP) as an index of improved cardiac function.

Another clinical trial indicates apples as a source of compounds which can be associated with source of reducing risk of cardiovascular disease (CVD) (Kroon 2016). The effect of the ingestion of epicatechin-rich flavanol extract and isolated apple procyanidins on systolic blood pressure (BP) and other cardiometabolic biomarkers was studied in the cited paper. Low epicatechin and procyanidin doses, high epicatechin and procyanidin doses, high procyanidin only and as control, no epicatechin, and procyanidin were administrated for a group of 42 participants. The results of the study showed that none of the isolated flavanol treatments significantly changed systolic or diastolic BP (peripheral and aortic), plasma nitric oxide (NO) reaction products, or measures of arterial stiffness, suggesting that, in isolation, neither monomeric flavanols nor PCs affect BP, blood lipid profiles, endothelial function, or glucose control.

Some studies demonstrated that moderate daily consumption of wine is associated with a lower risk of coronary heart disease. Khan et al. (2015) described the effects of oligomeric procyanidins (OPCs) on vascular endothelial function. They provided an explanation for the reduced incidence of coronary heart disease in red wine drinkers. OPCs were proved to induce atheroprotective changes in vascular function through oxidant signaling mechanisms originating from the mitochondrial electron transport chain.

The efficacy of procyanidins obtained from grape seed extract to decrease blood pressure was investigated by Belcaro et al. (2013). In this study, a controlled group formed by 119 healthy pre- and mildly hypertensive subjects were involved. After 4 months of treatment with two dosages of procyanidins (150 and 300 mg/day), microcirculation state and plasma oxidative status were evaluated. The treatment had beneficial cardiovascular effects that complement current intervention strategies in the hypertension area. The effect on blood pressure (decrease of blood pressure, decrease of the diastolic pressure) and oxidation of membrane lipids (endothelial dysfunction, formation of oxidized LDL, and activation of phagocytic cells) were

observed. The compounds improved endothelial function and promoted microcirculation decreasing the plasma oxidative status.

Valls et al. (2016) reported the effects of Oligopin® (extract from French maritime pine bark with low molecular weight procyanidins) in a clinical trial formed by 24 participants on cardioprotective effects. The randomized patients received a placebo 2 times a day or Oligopin® quantified extract also 2 times a day for 5 weeks each. The measured parameters were blood pressure, lipid profile, anthropometric variables, and other CVD risk biomarkers. The consumption of Oligopin® improved lipid cardiovascular profile and represents one of the scarce ways to increase HDL-c in stage-1-hypertensive subjects. In addition, it also tends to improve systolic BP and LDL oxidation.

40.5.3 Diabetes

Diabetes is a disease characterized by the increase of blood glucose levels. Gonzales-Abuin et al. (2015) suggested that procyanidins help in the hemostasis of glucose in various tissues, reducing the lipogenesis and modulating the secretion of insulin in pancreatic cells. Sun et al. (2016) reported proanthocyanidins as retina protector against early diabetic injury by activating the Nrf2 pathway. Even though the exact mechanisms involved remained unclear, the authors present several previous studies suggesting mechanisms responsible for the protective effect of grape seed procyanidin extracts.

Vanschoonbeek et al. (2006) showed no significant reduction in glucose values using cinnamon diet in postmenopausal type 2 diabetes patients, finding no time × treatment interactions for whole-body insulin sensitivity or oral glucose tolerance, nor changes in the blood lipid profile of fasting subjects following cinnamon supplementation. At the same time, the study of Mang et al. (2006) on the effects of cinnamon aqueous extract in adult diabetes patients on oral hypoglycemic treatment revealed a moderate decrease of fasting plasma glucose, but no effect on glycated hemoglobin A1c, serum lipids, or blood coagulation parameters.

Akilen et al. (2010) studied the effect of cinnamon administered daily (2 g) over a period of 12 weeks in a placebo-controlled double-blind clinical trial. The study demonstrated a significant reduction in blood pressure, fasting plasma glucose, and body mass index, suggesting the potential of cinnamon supplementation in a daily diet to regulate blood glucose and blood pressure levels.

Anderson et al. (2015) demonstrated a significant decrease of fasting insulin, glucose, total cholesterol, and LDL cholesterol and the increase of insulin sensitivity at the end of a 2 months trial after treatment with 500 mg cinnamon water extract daily.

40.5.4 Urinary Infections

Several clinical trials describe the effect of procyanidins on urinary infections. In a randomized controlled 12-month follow-up trial, Kontiokari et al. (2001) described

the effect of administration of 50 ml of cranberry-lingonberry juice concentrate daily for the reduction of urinary tract infection recurrence. The results showed a 20% reduction in absolute risk in the cranberry group compared with the control group (16% recurrence in the cranberry group, compared with 36% recurrence in the control group).

The study of Foxman et al. (2015) evaluated the therapeutic effect of cranberry juice capsules in preventing urinary tract infections (UTI) after surgery. The results showed a decrease by half of the UTI for women undergoing elective benign gynecological surgery involving urinary catheterization (19% vs. 38% for the control group).

The trial developed by Wan et al. (2016) studied the protective effect of highly concentrated cranberry juice against urinary tract infections, using 55 uncircumcised boys and 12 circumcised boys, from 6 to 18 years old, with histories of urinary tract infections. The results showed that prophylactic treatment with cranberry juice led to the reduction of bacteriuria incidence (especially *E. coli*) to 25%, compared with the negative control group (37%) and positive control group (33.3%). The protective effect of the cranberry juice was assigned to its proanthocyanidins content.

40.5.5 Other Diseases

Rumex acetosa L. (garden sorrel), known as a plant with high content of oligomeric and polymeric proanthocyanidins and flavonoids, was used in the clinical trial NCT02039648 in order to establish the prophylactic potential as mouth rinse on periodontitis (especially on *Porphyromonas gingivalis*, one of the major pathogens associated with the onset and progression of periodontitis) (Beikler 2014). Changes of the intraoral prevalence of *Porphyromonas gingivalis*, Approximal Plaque Index and Sulcular Bleeding Index were observed from baseline to 7 and 14 days; changes of cytopathological appearance of the mucosal tissue were observed from baseline to 7 days.

Heinrich et al. (2006) evaluated the contribution of procyanidins-rich cocoa to endogenous photoprotection. Their results suggested that a 12 weeks diet containing high doses of procyanidins (having as main compounds the monomers epicatechin – 61 mg/day and catechin – 20 mg/day, total procyanidin oligomers – 247 mg) provided photoprotection against UV-induced erythema, significant decrease of skin roughness and scaling.

The procyanidin mixture Pycnogenol® (extracted from pine tree bark) was evaluated for application in treating *attention deficit hyperactivity disorder* (ADHD). The product was reported to decrease the hyperactivity and impulsivity in a one-patient study (Heimann 1999). The study of Trebaticka et al. (2006) on 61 patients with ADHD found beneficial effects of Pycnogenol® at a dose of 1 mg/kg and an administration period of 1 month. A very interesting conclusion of the study of Trebaticka et al. was that the effect was sex-related (being more effective in boys). Administration of Pycnogenol® caused a significant reduction of hyperactivity, improved attention and visual-motoric coordination, and concentration of children

with ADHD. At the same time, addition of Pycnogenol® to the classic ADHD treatment with dextroamphetamine resulted in superior effects.

40.6 Application in Food (Including Correctly Cooking Foods Rich in Phytochemicals)

Proanthocyanidins in general (among which procyanidins are the most common) can be found in a series of foods, such as fruits (avocado, bananas, blackberries, grapes, plums, etc.), vegetables (broad beans, etc.), nuts (almonds, peanuts, pistachios, etc.), grains (barley, buckwheat, red beans, etc.), spices (cinnamon, curry, etc.), and beverages (beer, grape juice, tea, wine, etc.). A very useful database regarding the procyanidin content of different foods is maintained by the United States Department of Agriculture (Haytowitz et al. 2018). Table 6 presents the content of proanthocyanidins in common fruits and vegetables.

Several procyanidin-rich products are also commercially available. Cranberry extract powder is considered as food supplement which can be used in fruit-flavored and isotonic drinks, tea drinks, vitamin-enhanced waters, and yogurts. Grape seed powder also exists on the market. It which can be added to food directly, used in cooking and baking, diluted in water or other drinks, or used as an ingredient in more complex products such as protein powders, dietary supplements, functional foods and drinks, and sports nutrition. Another source rich in procyanidins is cocoa; it can be used as powder in cookies, chocolate, etc., and unheated and untreated at temperatures allow it to keep its nutrient-dense qualities.

Due to their antimicrobial and antioxidant properties, procyanidins could be successfully applied for extending the shelf lives of different meat products (Jeong et al. 2015). The cited study presents the effect of procyanidins (extracted from grape seeds, no further details on composition provided) on samples of pork patties: the samples had higher redness and yellowness values, as well as lower registered values for volatile basic nitrogen, 2-thiobarbituric acid reactive substance, and total bacterial counts assays.

The correct cooking of foods rich in procyanidins represents the subject of only a few research papers. Generally speaking, the processing step reduces the higher procyanidin content. Rodriguez-Mateos et al. (2014) showed that the thermal treatment has more pronounced effect on the higher procyanidins (nonamers and decamers disappeared, in the same time, the dimers and trimers having increasing values), with no significant changes in the total procyanidin content. Similar behavior was observed after extrusion of freeze-dried blueberry pomace at 180 °C or after processing berries into juiced, canned, and pureed products.

The study of Stahl et al. (2009) on the influence of preparation of cocoa-containing food on recovery of PAs revealed that the temperature was not the key factor in procyanidin loss but the leavening agents used. Comparing the effect of

Table 6 Maximum total proanthocyanidins (PAs) content in common foods (monomers not presented) (Gu et al. 2001; Haytowitz et al. 2018)

No	Fruit/vegetable	Max. PAs content (mg/100 g edible portion)					
		Dimers	Trimers	4–6mers	7–10 mers	Polymers	Total
1	Apples, different varieties, with peel, raw	105.81	23.2	49.5	39.26	59.93	277.7
2	Apples, different varieties, without peel, raw	79.55	7.88	26.71	22.51	33.75	170.4
3	Blueberries, raw	9.0	6.8	26.56	32.32	260.4	335.08
4	Cranberries, raw	33.16	63.62	163.38	118.29	2181.79	2560.24
5	Blackberries, raw	9.5	5.84	14.56	10.76	6.04	46.7
6	Chokeberries, raw	12.5	10.3	40.32	52.9	1990	2106.02
7	Raspberries, raw	40.6	13.92	15.21	4.39	–	74.12
8	Strawberries, raw	8.7	9.4	38.95	28.84	97.81	183.7
9	Cherries, sweet, raw	4.8	4.9	7.74	2.03	–	19.47
10	Green grapes, raw	2.9	2.1	8.68	9.9	79.18	102.76
11	Red grapes	5.26	1.64	7	7.44	54.31	75.65
12	Grape seeds, raw	3197.7	687.5	–	–	–	3885.2
13	Grape skins, raw	82.95	18	–	–	–	100.95
14	Peaches, raw	25.2	6.94	26.25	17.1	34.54	110.03
15	Pears, raw	4.33	3.36	8.96	8.02	56.33	81
16	Plums, raw	74.02	31.16	75.7	54.48	98.71	334.07
17	Apricots	23.61	42.10	–	–	–	65.71
18	Kiwis, gold	1.6	1.2	5	5	–	12.8
19	Avocados, raw	3.28	2.6	5.79	1.96	–	13.63
20	Mangos, raw	1.8	1.4	7.2	–	–	10.4
21	Bananas, raw	0.8	0.94	2.98	–	–	4.72
22	Sorghum, sumac bran	122.09	147.8	774.79	904.23	3384.67	5333.58
23	Sorghum, sumac whole grain	36.72	46.82	232.25	298.35	1385.26	1999.4
24	Pinto beans, raw	34.37	29.81	132.52	143.75	489.3	829.75
25	Small red beans	19.4	18.1	80	75.7	252.9	446.1
26	Red kidney beans	26.4	29.1	117.7	105.3	263.4	541.9
27	Hazelnuts	17.73	17.09	85.57	102.69	442.95	666.03
28	Pecans	49.46	28.77	119.79	99.54	297.31	594.87
29	Pistachios	15.1	12	47.26	43.28	158.74	276.38
30	Baking chocolate, Unsweetened	217.38	186	563	366	830	2162.38
31	Dark chocolate	128	108	454	295	697	1682
32	Cinnamon, ground	256.29	1252.2	2608.63	1458.32	2508.78	8084.22

baking soda (sodium bicarbonate) and baking powder (mixture of baking soda and acidic ingredients) used as leavening agents, the authors showed that the key factor in procyanidin loss was represented by the pH level (higher recoveries of procyanidins being recorded at lower pH values reached using baking powder).

The literature data suggests that, due to the reduction of higher procyanidins on processing, the consumption of raw products it is recommended, if possible.

40.7 Safety: Toxicity and Side Effects

The intake of PAs can be beneficial for different diseases, but the most important is to find the optimal doses at which the biologic compounds are safe and have no adverse reactions. Ottaviani et al. (2015) investigated the effects of cocoa flavanols (CF) (including procyanidins) intake amount and intake duration on blood pressure, platelet function, metabolic variables, and potential adverse events. The limitations of their study lie in its duration and the number of volunteers studied; however, due to the lack of the studies on human subjects (up to 2015), the authors suggested that their study provides relevant and needed information for current safety assessments. The intake of cocoa flavanols (including procyanidins) up to 2000 mg/day for 12 weeks was well tolerated in healthy adults; the consumption was not associated with significant changes in blood pressure or platelet function.

Safety assessments of grape seeds extract (rich in procyanidins) have been conducted using animal models by Ray et al. (2001) and Yamakoshi et al. (2002a). Ray et al. (2001) conducted acute oral toxicity, dermal toxicity, dermal irritation, and eye irritation studies administrating doses of PAs from grape seeds extract. LD_{50} was found to be greater than 5000 mg/kg in albino rats via gastric intubation and greater than 2000 mg/kg when administrated dermal. The examination of other organs like the brain, duodenum, heart, kidney, liver, lung, pancreas, and spleen did not reveal any abnormalities. The serum chemistry was also not changed in female mice at chronic administration. The acute and subchronic oral toxicity of PAs from grape seeds extracts was also performed on Fischer 344 rats (Yamakoshi et al. 2002a). The authors evaluated mutagenic potential by the reverse mutation test using *Salmonella typhimurium*, the chromosomal aberration test using CHL cells, and the micronucleus test using ddY mice. No signs of toxicity were found indicating the safety of preparation. Lluís et al. (2001) confirmed the previous results describing LD_{50} of PAs from red grape marcs (variety Syrah) as being higher than 5000 mg/kg.

Due to the lack of consistent data regarding evaluation of the safety and tolerability of continuous intake of oral grape seeds extract, Sano (2017) conducted a 4-week toxicity test with daily doses of 1000–2500 mg proanthocyanidin-rich grape seeds extracts in healthy Japanese volunteers. Measured physical parameters as body weight, blood pressure, and pulse rate showed that tested doses were well tolerated, and subject compliance was 100%.

The potential systemic toxicity of Oligopin® was evaluated by Segal et al. (2018). The preparation was showed to be not acutely toxic via oral administration at up to 2000 mg/kg and was well tolerated following repeated oral administration to

Sprague Dawley rats, with a NOAEL (no-observed-adverse-effect level) of 1000 mg/kg/day.

Cranberry extract powder is considered a food supplement and that's why it was emitted an opinion pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. None of the clinical trials conducted on this product indicated adverse reactions. The Panel (Turck et al. 2017) which analyzed the safety of this product took into consideration the studies of Cos et al. (2003), and Prior and Gu (2005), regarding absorption, distribution, metabolism, and excretion of this biologically active compounds, and some clinical studies on human subjects (Valentova et al. 2007).

The no-observed-adverse-effect level (NOAEL) of a chronic toxicity study of procyanidins from edible plant extracts is different according to the study or clinical trial which is reported.

The analytical standards available have safety data sheets, covering some aspects regarding their toxicity. For example, the monomers catechin and epicatechin have oral LD_{50} values (mouse and rats) >10,000 mg/kg and 1000 mg/kg, respectively, while for the dimers, trimers, and higher procyanidins, there are no acute toxicity data available. The GHS (Globally Harmonized System of Classification and Labelling of Chemicals) hazard statements usually presents the hazard statements H315 *Causes skin irritation*, H319 *Causes serious eye irritation*, and H335 *May cause respiratory irritation* and several precautionary statement codes for the monomers and other available procyanidins (e.g., Procyanidin B2, Procyanidin B3, etc.). The hazards and precautionary statements should be read carefully and understood, while the general safety regulations in the laboratory should be respected when working with procyanidins fractions or purified procyanidins.

40.8 Marketed Products

Several products based on PAs are present on the market. These are mainly herbal drugs (containing extracts), herbal products, dietary supplements, and functional foods: Pine Bark Extract 95%®, Enovita®, Oligopin®, Pycnogenol®, Leucoselect®, Endotélon®, Masquelier's®, Anthogenol®, Enzogenol®, etc.

Enovita® is a food-grade grape seed proanthocyanidin extract (seed extract from *Vitis vinifera* L.) specifically designed for the food market, developed by Indena (Milan, Italy), with a content more than 95.0% of proanthocyanidins determined by spectrophotometry, 5.0–15.0% of catechin, and epicatechin determined by high-performance liquid chromatography (declared by the company). Belcaro et al. (2013) developed a registry study on 19 healthy, pre- and mildly hypertensive subjects regarding the efficacy of a standardized grape seed procyanidins extract (GSPE, Enovita®) to decrease blood pressure when associated with nondrug intervention. According to Belcaro report, Enovita contains ca. 8.6% monomeric procyanidins (catechin, epicatechin, and epicatechin gallate) and ca. 91% proanthocyanidins (OPC), of which 9% are of the dimeric type. A decrease of systolic

blood pressure was observed at month 1, but the decrease was significantly higher in the treatment group.

French Maritime Pine Bark extract (FMPBE) rich in procyanidolic oligomers was tested as Oligopin® (PureExtract, DRT, France) in different clinical trials or studies on animals, where the systemic toxicity and mutagenicity were evaluated. Segal et al. (2018) have evaluated systemic toxicity of Oligopin® (obtained from the pine tree *Pinus pinaster*) in an acute oral limit test and a 90-day repeated dose oral toxicity study with Sprague Dawley rats. The researchers assessed potential mutagenicity in a bacterial reverse mutation assay and *in vitro* mammalian chromosome aberration assay with human lymphocyte. The results of their tests indicate that Oligopin® was nongenotoxic in both bacterial and human cell assays, was not acutely toxic via oral administration at up to 2000 mg/kg, and was well tolerated following 90 days of oral administration to rats, with a no observed adverse effect level of 1000 mg/kg/day. The composition of the recipe used is procyanidolic oligomers, out of which dimers constituted 15–20%, trimers 15–20%, and tetramers and higher oligomers 30–40%.

The effect of Oligopin® consumption on blood pressure of randomized group of 24 people with mild/moderate degree of hypertension was evaluated in the clinical trial NCT02063477 conducted by Technological Center of Nutrition and Health, Spain (Valls and Sola 2014). The differences in the time of evolution of blood pressure in both two arms of intervention were observed. Other measured parameters were endothelial function, biomarkers related with endothelial function, and biomarker related with inflammatory processes.

Poussard et al. (2013) reported that heat-shock protein beta-1 (HSPB1) is modulated by Oligopin®. Oligopin® prevented the stress-induced phosphorylation of HSPB1 and its subsequent structural modification. This supports the therapeutic usefulness of preparation for preventing the age-related muscle mass loss and for protecting muscle cells from oxidative stress. Valls et al. (2016) reported Oligopin® as being characterized by a practical absence of other tannins (<1%) and a high content in low molecular weight oligomeric procyanidins (OPCs >70%; dimers about 20%).

Pycnogenol® (Horphag Research Ltd., UK, Geneva, Switzerland) is a standardized extract with many benefices for human health due to its chemical composition. It is a standardized plant extract obtained from the bark of the French maritime pine *Pinus pinaster* Aiton (formerly known as *Pinus maritima*), subspecies *Atlantica des Villar*. With a reach composition in procyanidins comprising of catechin and epicatechin subunits with varying chain lengths, it is now utilized throughout the world as a nutritional supplement and as a phytochemical remedy for various diseases ranging from chronic inflammation to circulatory dysfunction, including several impaired psycho-physiological functions (D'Andrea 2010). This supplement is good in the treatment of attention deficit hyperactivity, osteoarthritis, type 2 diabetes, and cardiovascular diseases. Pycnogenol® can be used to slow the aging process, maintain healthy skin, improve athletic endurance, and improve male fertility. Jessberger et al. (2017) reported cellular pharmacodynamic effects of Pycnogenol® in randomized controlled pilot study with 33 patients with severe osteoarthritis. Grether-Beck et al. (2015) reported Pycnogenol®'s effects on human

skin (photoprotection, reducing hyperpigmentation, improving skin barrier function, as well as extracellular matrix homeostasis).

Leucoselect® is a standardized extract from grape seeds developed by Indena (Milan, Italy) with a content of 95.0–100% of proanthocyanidins, determined by spectrophotometry, and catechin and epicatechin between 13.0 and 19.0% evaluated by HPLC. Nuttall et al. (1998) reported Leucoselect® as a placebo treatment in a clinical trial aiming to evaluate the effects of a capsule formulation of an antioxidant polyphenolic extract of grapes on serum total antioxidant activity and vitamin C and E levels. As results they reported that the extract had no effect on serum vitamins C and E levels but increased total serum antioxidant activity. To further improve their bioavailability, Leucoselect® has been formulated with soy (non-GMO) phospholipids (1:3 w/w), thus generating Leucoselect® Phytosome®.

Several other extracted from lychee fruits and grape seeds rich in PAs are currently commercialized under different names (Oligonol®, Vitaflavan®, MegaNatural™).

Besides those marketed products, several foods and beverages with high PAs content are available (as previously presented in Sect. 6) and could provide the necessary daily intake of PAs for a healthy and equilibrated diet (Bhagwat and Haytowitz 2015).

40.9 Patents

One of the first patented recipes based on PAs was Jack Masquelier patent (patent number 4.698.360 from 1987) "Plant extract with a proanthocyanidins content as therapeutic agent having radical scavenger effect and use thereof," where the author's invention relates to the use of a plant extract with a PAs content as therapeutic agent with radical scavenging effect as well as the use of pharmaceutical compositions containing this extract as active ingredient. The recipe is based on bark conifers (*Pinus maritime* Lam.) rich in biological active compounds.

The invention of Soulier et al. (2012) relates to the process of extraction involving the use of cranberries (*Vaccinium macrocarpon*) as a raw material, crushed fruits, or already pre-purified extracts rich in PAs. This invention allows to obtain extracts containing more than 10% PAs, using hydroalcoholic extraction and absorption on a macroreticular polymeric resin. Venkatramesh et al. (2013) describe in their patent the production and extraction of procyanidins from plant cell cultures of Theobroma or Herrania. Schmitz et al. (2017) patented "Compositions and methods of use of A-type procyanidins." The authors extracted procyanidins from peanut skin and investigated characterized compounds for their effect on nitric oxide (NO) production and vasorelaxation using serum-free human umbilical vein endothelial cell (HUVEC) culture system *in vitro* and rabbit aortic ring *ex vivo* models. Additionally, the effects of A1 dimer on platelet count in whole blood were measured. Hammerstone and Chimel (2003) patented the method of extraction of procyanidins from cocoa. They used defatted, unroasted, or unfermented cocoa beans and optimized extraction parameters (solvent used, extraction temperature, extraction pH) in order to obtain

higher yields of PAs. A preferred extraction technique was countercurrent extraction chromatography. The patent of Howard et al. (2012) described a method of extraction of PAs from any plant pomace (apples, pine bark, cinnamon, cocoa, grapes, bilberry, black currant, green tea, black tea, chokeberry, blueberry, and sorghum) by alkaline hydrolysis. Subsequently the active compounds can be used in dietary supplements or added to products to enhance health benefits. The invention of Rull et al. (2004) related to vegetable extracts containing at least 15 and preferably 20–25% of OPC of A2 type. The authors patented the isolation of the compounds from litchi fruits shell involving extraction with methanol and liquid chromatographic purification step.

Patent describing preparations containing A-type procyanidins and their derivatives for treatment or prevention of certain tumors/cancers (especially tumors/cancers overexpressing cycloxygenase-2 (COX-2)) can also be found (Schmitz and Kwik-Uribe 2007). Rohdewald and Ferrari (2003) patented the use of proanthocyanidins and arginine in the treatment of erectile dysfunction. The patent obtained by Danoux et al. (2002) described the use of procyanidin oligomers in the field of cosmetics for skin treatment products, especially products with the ability to counteract skin aging effects.

Several other patents referring to commercial products are described in Sect. 8.

40.10 Perspectives

In order to cope with an increasing global population and a quantity of wastes obtained from agricultural industry, to conform to the principles of sustainable development and bioeconomy, it is a *must* to find new methods to obtain added value products from plant waste.

This is the case of plants and fruits with a high amount of biological active compounds, as procyanidins. Based on the United Nation Food and Agriculture Organization (FAO) statistics, at the end of the year 2016 (most recent available), huge productions of plants rich in procyanidins, which processed will produce waste, were reported worldwide: cocoa beans, 4,466,575 t; grapes, 92,281,609 t; tea, 8,368,892 t; strawberries, 12,920,201 t; raspberries, 795,249 t; nuts, 1,087,750 t; cranberries, 683,672 t; cocoa beans, 4,466,575 t; cinnamon, 300,630 t (from 13 countries); apples, 133,777,757 t; apricots, 3,955,025 t; etc. The growing demand of green materials and renewable resources is due to the fact that wastes from agroindustry can be recycled into food/feed/regenerable fuels, fertilizers, pharmaceutical, and cosmetic products. All over the world, the pomace remaining after fruits extraction is produced. It is not valued as highly profitable waste, but instead leads to several environmental and waste disposal problems.

Also, in the present *eco* and *bio* rush, materials such as essential oils, pharmaceuticals, supplementary foods, cosmetics, etc., are obtained from plants. From this perspective, the use of active compounds obtained from plant waste is encouraged, especially that agro-horticultural waste is cost-effective natural sources. Initiatives regarding the valorization of plant wastes are developed channeling on different types of compounds or plants pomace. Kolodziejczyk et al. (2007) considered by-

product deriving from apple juice pressing as a source of dietary fiber (about 50% of dry weight) and phenolics (from 1200 to 4000 mg/kg dry weight), including flavanols (catechin, epicatechin, procyanidins), hydroxycinnamates, and dihydrochalcones. Altiok et al. (2007) investigated the possibility of recovering of proanthocyanidin from the by-product of Turkish traditional product (*molasses*). Teixeira et al. (2014) presented in their review paper the types of residues produced by the wine industry and the types of compounds that can be obtained from grape waste. Being one of the most important and widespread agro-economic activity, grape industry produces organic residues (nine million tons/year) and inorganic one (diatomaceous earth, bentonite clay, and perlite).

Nevertheless, implementation of waste management from agro-industry is a challenging task, making the development of innovative and effective capitalization procedures necessary in order to recycle, reuse, and recover these residues. Food wastes should no longer be regarded as a waste to be disposed, but as a renewable source of valuable molecules that should be fully exploited.

Adequate procedures to obtain the target compounds will provide successful achievements for phytochemical recovery and obtaining added value products. At the same time, scientific advances are necessary toward the separation and purification of procyanidin compounds, as well as for the *in vitro* and *in vivo* evaluation of the potential effects of those compounds. Moreover, supplementary scientific data are necessary to support the various claims of beneficial effects of regarding procyanidins arising either from folk medicine or from industrial companies in the area of food supplements and related products.

40.11 Cross-References

- ▶ Antioxidants in Diets and Food
- ▶ Introduction of Phytonutrients
- ▶ Sesquiterpenes in Cereals and Spices
- ▶ Stilbenoids in Grapes and Wine
- ▶ Tea Catechins

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Pyrazines in Food

41

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Abstract

Pyrazines are volatile compounds that share a common chemical structure, including a monocyclic aromatic hydrocarbon with a counterpoint nitrogen atom. Pyrazines can be synthesized chemically or biologically and can be used as flavor additives. Pyrazines are mainly formed during food thermal processing, such as coffee, cocoa, roasted nuts and seeds, cereals and cereal products, meat products, wine, and so on. Pyrazines are key flavors in the development of roasted foods. Their formation is closely related to the Maillard reaction, which involves the reaction between the α-dicarbonyl and amino acid groups to yield an aminoketone.

Keywords

Pyrazines · Roasted food · Thermal processing · Bioactivity

41.1 Introduction

Pyrazines are volatile compounds that share a common chemical structure, including a monocyclic aromatic hydrocarbon with a counterpoint nitrogen atom. Pyrazines can be synthesized chemically or biologically and can be used as flavor additives. Pyrazines are mainly formed during food thermal processing (Asikin et al. 2016). Pyrazines are key flavors in the development of roasted foods. Their formation is closely related to the Maillard reaction, which involves the reaction between the α-dicarbonyl and amino acid groups to yield an aminoketone. It suffers further condensation producing new heterocyclic compounds as the pyrazines. Several factors including the composition of food matrix may affect pyrazine formation. Moreover, heating parameters such as temperature and time are probably the most influential factors. The interests of food industry in pyrazines began in the 1960s and continue nowadays. The continuous increasing numbers of publications about pyrazines were indicated.

41.2 Pyrazines in Foods

41.2.1 Coffee

Coffee is an evergreen shrub or small tree of Rubiaceae. Its production is mainly concentrated in tropical developing countries at Latin America, Africa, and Asia, such as Brazil, Colombia, India, Liberia, Ethiopia, and Vietnam (Zhou and Ren

2016; Zhu and Jiang 2010; Sun and Xiong 2010). Coffee produced around the world has its own characteristics: Brazilian coffee, moderately bitter, with gentle flavor and unrestrained tropical taste, it is an excellent base for mixed coffee; Colombian coffee, smooth sour and sweet flavor, rich and full-bodied; Mandening coffee, the aroma is thick and thick; and Yunnan small grain coffee, strong but not bitter, fragrant but not strong, and slightly sour fruit taste (Zhou and Ren 2016; Zhou and Chen 2010). Aroma components of coffee are mainly produced in the process of coffee roasting. In the process of roasting, Millard and caramelization reactions mainly take place, producing aroma volatile components (Zhou and Ren 2016). The Millard reaction is the reaction of hydroxyl and amino groups. For raw coffee beans, it is mainly the reaction of proteins, amino acids, and sugars (Ren and Zhou 2018; Baggenstoss et al. 2008). There are differences in these ingredients in raw coffee beans, but not much, so there is little difference in the Millard reaction that takes place during the roasting process. The Millard reaction has the same reactants and the same products under the same conditions. The difference in content and composition is mainly due to the degree of baking, that is, the degree of reaction (Moon and Shibamoto 2009; Kumazawa and Masuda 2003; He et al. 2015). Since the beginning of the twentieth century, the chemical composition of coffee, especially for coffee bean flavor chemicals from a lot of studies, has been reported. There are nearly more than 1000 kinds of volatile chemicals have been found in coffee bean extract. It found that about 400 kinds of compounds are heterocyclic compounds, including pyrrole, furan, thiazole, pbo, thiophene, imidazole, and pyrazine compounds (Buffo and Claudio 2004; Lv et al. 2015). As the roasting temperature raises, volatile compounds such as pyrazines, aromatic aldehydes, phenols, furans, pyrrole, and imidazole increase and change the characteristic aroma of coffee (He et al. 2015). Coffee beans with different degrees of roasting contain their own volatile substances, which lead to different degrees of roasting with different characteristics of aroma. Pyrazines are the main volatile substances in extremely shallow, shallow, and moderate roasts, while furans, pyrrole, and ketones play a major role in medium and medium deep roasts, and phenols and pyridines play a major role in deep, extremely deep, and French heavy roasts (Wang et al. 2018). Guaiacol has a smoky flavor, pyridine has a scorch flavor, pyrrole has a nutty and roasted flavor, furans can produce a pleasant scorch flavor, phenols are mainly manifested as pleasant clove flavor, and pyrazines and acids are mainly nutty and sour (Lv et al. 2015).

41.2.2 Cocoa

Cocoa (*Theobroma cacao* L.) is used as a raw material in many products and highly appreciated for its flavor. The compounds involved in the chocolate aroma vary accordingly to the cocoa variety are volatile acids, terpene and non-terpene alcohols, and aldehydes. Esters are detected in higher amounts in Criollo beans, which show a richer and peculiar aroma profile. It has been known that the compounds that have given cocoa and chocolate their flavors are N- and O-containing molecules such as

pyrazines, pyrroles, furans, aldehydes, and amides, which are mainly generated during the Maillard reaction (Ducki et al. 2008). The characteristic aroma of cocoa is mainly the aroma of roasted nuts produced by pyrazines and the aldehyde (Sylvie et al. 2008; Yue et al. 2012; Xiao et al. 2012). Pyrazines, especially 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 2,3,5,6-tetramethylpyrazine, are the main sources of cocoa aroma (Xiao et al. 2012; Christine et al. 2002; De Brito and Narain 2003). Even though the content of pyrazines in cocoa powder is very low (10^{-5}–10^{-4} g/L), it has an important contribution to the flavor of cocoa due to its extremely low flavor threshold. Flavor formation in cocoa is related, partly to thermal treatment (roasting) during which several modifications occur, including the Maillard reaction, which is mainly responsible for the accentuated decrease in the concentration of reducing sugars and amino acids during roasting. In roasted cocoa, volatile compounds are dominated by alkyl-substituted pyrazines followed by aldehydes and esters (Gill et al. 1984; Oberparlaiter and Ziegleder 1997; Ziegleder 1991). Among the different classes of volatile compounds present in cocoa products, pyrazines are by far the most studied. Due to their pronounced formation during roasting, pyrazines are used as index compounds of the roasting process (Hashim and Chaveron 1994; Jinap et al. 1998). The roasting process is a critical step for the development of the chocolate flavor from the precursors which are already formed during the fermentation and drying phases (Perego et al. 2004; Carro et al. 2015). Pyrazines, the dominant compounds which are responsible for the typical chocolate aroma, are mainly developed during this phase. The roasting process consists of a hot air treatment of the seeds in a temperature range between 110 °C and 140 °C. The higher temperatures lead to the over-roasted products with loss of the pleasant characteristics of cocoa and burnt taste (Perego et al. 2004). The roasting phase causes an increase in the content of alcohols, aldehydes, and pyrazines, while the other chemical compounds have less influence (Crafack et al. 2014).

41.2.3 Roasted Nuts and Seeds

Nuts as one type of non-splitting dried fruit of angiosperms have hard or tough walls and usually contain one seed. The research on the aroma substances of nuts can be traced back to the 1970s. Baking is an important food processing process, which can improve the flavor, color, texture, and appearance of food. The special flavor of nuts can only be obtained after the hot processing. There are the complex series of reactions among nonvolatile flavor precursor substances in tissues, which are mainly Millard reaction and thermal degradation reaction of lipids. Organic compounds such as pyrazine, furan, and pyrrole generated generate unique flavor, while unprocessed plant seeds have little or no fragrance (Li et al. 2011). The aroma of baked nuts is complex, including most volatile organic compounds, such as hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones, furans, pyridines, pyrazines, pyrrolids, thiazoles, thiophene, and the other sulfur compounds (Yu et al. 2017). The contribution of nitrogen, oxygen, and sulfur heterocyclic compounds to baking flavor is very important, many of which are formed

through the interaction between Millard reaction and lipid degradation products (Castro-Muñoz 2019). Pyrazines are considered as the main flavor compounds that present the typical roasted nut aroma and can be used as an important index to optimize the roasting conditions of nuts. Among them, 2,5-dimethylpyrazine has malt flavor and chocolate aroma, which may be the best compound to predict the flavor of roasted peanuts, hazelnuts, and so on (Braddock et al. 1995; Yu et al. 2017; Michael et al. 2016). Pyrazines can be detected in roasted hazelnuts, while pyrrolidine is not contained in raw kernels, and 2,5-dimethylpyrazine is the highest content (Alasalvar et al. 2003). Pyrazines provide roast hazelnuts with nutty, roasted, and sweet flavors. Among them, the barbecue-flavored 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine have a high dilution factor (Michael et al. 2016). The methoxypyrazine-containing, such as 2-isobutyl-3-methoxypyrazine and 2-butyl-3-methoxypyrazine, can be detected in roasted peanuts and has a strong pea-like odor (Braddock et al. 1995; Sabine et al. 2004; Irene et al. 2008). Shimoda et al. (1997) compared the differences in volatile flavor components of deep-baked and light-baked sesame oil. The degree of baking increases the relative reduction of monoalkylpyrazines and the relative increase of dialkyl- and trialkylpyrazines. 1H-Pyrrole-2-carbaldehyde is the highest content of azoles and is the only reduction in deep baking. 4,5-Dimethylthiazole, 2-propyl-4-methylthiazole, and 2-butyl-5-methylthiazole were relatively increased in deep baking. And the content of guaiacol and 2-furyl mercaptan was significantly increased (Li et al. 2011; Sabine et al. 2004).

41.2.4 Cereals and Cereal Products

In addition to the inherent pyrazines in cereals, the most important source of pyrazines in cereal products is the Millard reaction, especially in thermal processing. Ammonia released from deamination of cereals protein also promoted the generation of pyrazines, such as wheat protein (Eon Lee et al. 2012). Pyrazines in beer, and in baking products, are an important research direction for pyrazines in cereals and cereal products. The contribution of pyrazines to roasted flavor of cereal products is the main reason to study their formation (García-Lomillo and González-SanJosé 2019). The important role of pyrazines in beer flavor led to earlier pyrazines studies focusing on pyrazines in malt and baked barley products (Wang and Sakurai 1969). Plenty of pyrazine compounds were found in the essential oil extracted from caramel malt (Zou et al. 2014). GC-MS and sensory results have shown that pyrazines and aldehydes were the most dominant flavor components in malt and roasted barley (Kim et al. 1998). For different types of malt, different amounts and proportions pyrazines were formatted in kiln-drying. And brewing couleurs had relatively high amount of pyrazines (Wittmann and Eichner 1989). Twenty-one pyrazines and main methylpyrazines, including 2,3-, 2,5-, and 2,6-dimethylpyrazines and trimethylpyrazines, were isolated from roasted barley by Harding et al. (1977, 1978). They indicated that pyrazines contributed to the flavor of beer, and roasted barley has a better performance (Collins 1971; Harding et al. 1978). Acetylpyrazine, 2-methyl-3-ethylpyrazine, and 5-methyl-5H-cyclopenta[b]pyrazine

were contributed significantly to the crust flavor (Schieberle and Grosch 1987). Concentrations of 13 pyrazines were determined in aging bottle beer, and most pyrazines decreased quickly in the first 48 h. 2-Methylpyrazine, 2,6-dimethylpyrazine, and 2-ethyl-5-methylpyrazine have been increased in the latter half of storage. However, there were no significant changes in frozen beer. This may indicate some changes of beer flavor during storage (Qureshi et al. 1979). Isolated from English beer, a result shown that pyrazine, methylpyrazine, dimethylpyrazine, and cyclopentapyrazine were important flavor essence, and there was a similar result in other beer and whisky (Harding et al. 1977; Peppard and Halsey 1981; Viro 1984). Extrusion cooking with HTST method to green malt at 130–160 °C obtained more total pyrazines than conventionally processed malt. 2-Vinyl-5-methylpyrazine and 2-vinyl-6-methylpyrazine only formatted in extruded malt (Fors and Eriksson 1986). Pyrazines deeply influence the aroma of bread and other baking cereal products. 2-Acetyl pyrazine and 2-methyl-3-ethylpyrazine are important to crust bread aroma. 2-Ethyl-3methylpyrazine and methylpyrazine are the 2 most abundant pyrazines of total 13 pyrazines detected in different wheat breads, which significantly affect the flavor of breads (Cochrane et al. 1995; Fadel and Hegazy 1993). The volatile components of rye bread were identified including 2-acetylpyrazine, 2-methyl-3-ethylpyrazine, and 6,7-dihydro-5-methyl-(5H)-cyclopentapyrazine in the crust (Schieberle and Grosch 1983). The formation of pyrazine, 2-ethylpyrazine, 2-methylpyrazine, 2-ethyl-3-methylpyrazine, and 2-vinylpyrazine was promoted in fermentation of wholemeal bread crust with higher temperatures, which may lead to a change of flavor (Qhairul et al. 2015; Qhairul et al. 2016). Thermal processing of cereal products brings lots of benefits, including reducing microbial contamination and promoting positive flavor and improving process properties (Neill et al. 2012). Appropriate heat treatment temperature can promote positive flavor, for malt and barley; dry conditions promoted formation of pyrazines at 180 °C (Channell et al. 2010). Heat treatment at 100 °C can eliminate harmful ingredients in materials, but does not induce the formation of flavor substances. When the temperature reaches above 120 °C, pyrazines and other compounds form quickly and result in roasting flavor (Xu et al. 2017). Heat treatment before baking can improve the formation of pyrazines and lead to a strong flavor of baking (Gu et al. 2015). Besides heat treatment, sourdough also showed effects to 11 pyrazines and resulted in the flavor of bread (Pétel et al. 2017). Gluten-free bread showed a lack of flavor which was due to the lack of pyrazines, but an addition of sugars and amino acids before baking could solve the problem (Pacyński et al. 2015). However, heat treatment is not entirely beneficial, and it will also bring some negative effects. Acrylamide, one of the Maillard-reactions products as a toxic substance, it coupled to pyrazine formation, become a noticeable problem in cereal products processing in industry (García-Lomillo and González-SanJosé 2019). In recent studies, natural antioxidants such as phenolic compounds may prevent their binding, but it also led to a reduction of pyrazines until 81%, resulting in a negative effect to flavor of roasting food (Hedegaard et al. 2008; Jin et al. 2013; Mildner-Szkudlarz et al. 2017). The block of pyrazines is also observed in some processing method, such as vacuum frying potato chips, and some additives can also lead to the formation of unpleasant flavor substances (Belkova et al. 2018; Zhao et al. 2017).

41.2.5 Meat Products

Pyrazines make contributions to flavor notes such as cooked and roasted aromatic notes. This is the main role of pyrazines in meat products (García-Lomillo and González-SanJosé 2019). Different from cereals, there are not many pyrazines in the raw meat, but the thermal processing of the meat produces a lot of pyrazines. Several pyrazines and their derivatives are used in food processing for flavoring purposes or decreasing microbial contaminations (Schöck et al. 2018). Pyrazines derivatives are important flavor substances to meat products. In pork, beef, lamb, chicken, and other meat products, pyrazines are widely detected and confirmed as important odor components (Shahidi 1994). 2-Methyl-3-furanthiol is considered as the most important chemical compound for chicken flavor. Alkylpyrazines are important for fried and roasted chicken flavor, but not chicken broth (Jayasena et al. 2013). After heated at 160 °C for 10 min, methylpyrazines and trimethylpyrazines were identified in pork samples with added carbohydrates (Lauridsen et al. 2006). Thirty-three pyrazines were isolated from beef at 162.7 °C, and 9 alkyl-substituted pyrazines were found in shallow-fried beef as flavor components (Mussinan et al. 1973; Watanabe and Sato 1971). Lamb meat has a stronger flavor profile compared to goat meat because of a higher concentration of volatiles including pyrazines and other lipid-derived components. Besides the quality of raw meat, meat flavors were also influenced by the cooking procedure (Shahidi et al. 1986). Heat treatments, such as roasted and cooked, will produce lots of pyrazines derivatives and forms aroma. Due to different temperature profile, different pyrazines derivatives were formatted in different type of cooking methods. One hundred fifty volatiles were identified in pan-fried pork chops. They are mostly hydrocarbons and only a few nitrogen and sulfur compounds like pyrazines (Meinert et al. 2009). The formation of heterocyclic volatiles was influenced by temperature, compared with roasted pork steaks, and significantly higher (Meinert et al. 2007; Mottram 1985). Among all the cooking methods, the roasting is the one that produces the most variety and quantity of pyrazines. Microwave treatment may induce a rapid surface dehydration that promoted the formation of pyrazines. It produced much more methylpyrazines, 2,3-dimethyl-5-ethylpyrazine, and 2,5-dimethyl-3-ethylpyrazine. And boil will produce much more 2-ethyl-6-methylpyrazine and 2,3,5-trimethylpyrazine (MacLeod and Coppock 1976). Moreover, pyrazines have much higher content in electric oven-cooked pork loin than in oven and air fryer (Yang et al. 2016). For antimicrobial purposes, pyrazines derivatives have been studied and used in the industrial productions. *Paenibacillus polymyxa* could produce alkyl-substituted pyrazines as mediators of antimicrobial effects and works on many plant and human pathogens (Rybakova et al. 2016). In chicken meat processing, 5-isobutyl-2,3-dimethylpyrazine decreased 95% viable bacteria in a maltodextrin carrier, and 2-isobutyl-3-methylpyrazine could improve the antimicrobial effects by surface dehydration to meat (Schöck et al. 2018). The decontamination of eggshells can also be done with specific pyrazine derivatives and shown similar efficiency to traditional methods (Kusstatscher et al. 2017).

41.2.6 Wine

Aroma is very important to wine, influencing the quality of wine directly. As an important flavor substance, pyrazine has been studied for many years, and its role in the aroma of wine has been proved. The naturally occurring and added methoxypyrazines significantly influenced the aroma of wine. To Sauvignon blanc wines, a higher methoxypyrazine concentration was significantly different than the lower in aroma; an addition of 8 ng/L 2-methoxy-3-(2-methylpropyl)pyrazine to the methoxypyrazine-free wine could make a significant contribution to its vegetative aroma (Allen et al. 1991). An analysis of pyrazines in the 22 wines and 16 juice samples from different regions shown that 2-methoxy-3-(2-methylpropyl)pyrazine exist in all the samples and 2-methoxy-3-(1-methylethyl)pyrazine exist in 11 wines and almost all juice samples (Lacey et al. 1991). A quantitative analysis of methoxypyrazines to 18 red wines shown that methoxypyrazine mainly contribute to wine flavor, and 2-methoxy-3-(1-methylpropyl)pyrazine has a very low level in all wines (Allen et al. 1994). The level of 3-isobutyl-2-methoxypyrazine was correlation with vegetable aromas and anticorrelation with fruity aromas (Gracia-Moreno et al. 2014). The growing environment of the fruits used in wine making, including temperature, light, irrigation, and planting density, all affects the content of pyrazine in wine and ultimately contributes to our perception of flavor in finished wines (Lund and Bohlmann 2006). Fruits grown in cooler regions had higher methoxypyrazine levels (Lacey et al. 1991). Irrigated vines had significantly higher level of 3-isobutyl-2-methoxypyrazine than nonirrigated plants, and the average levels of 3-isobutyl-2-methoxypyrazine in vines with the higher plantation density were also higher (Sala et al. 2005). In wine volatiles, isobutyl methoxypyrazine was significantly influenced by harvest date and decreased as ripening progressed (Bindon et al. 2013). The pyrazines in wine are not entirely brought in by the fruits. Some of them are converted during the brewing process. The productions of methoxypyrazines are related to the methylation of nonvolatile hydroxypyrazine precursors. Two *O*-methyltransferases were confirmed to catalyze the methylation of hydroxypyrazines and finally influence the wine flavor (Dunlevy et al. 2010). Besides, Cabernet Franc ice wines were produced with yeast, and the change of yeast strain could lead to a change on the pyrazines concentration (Synos et al. 2015).

41.3 Pyrazine Formation and Main Influencing Factors in Processed Foods

Pyrazines, as the products of thermal food processing, are known to contribute to the unique flavor generation of the food (meat products, roasted products, cereal products). The intermediate product (α-dicarbonyl) of Maillard reaction is the most vital precursor substance of Strecker degradation which leads to the formation of pyrazine. The α-dicarbonyl can be generated by the rearrangement of Amadori or Heyns compounds, which comes from the combination of amino acids and reducing sugars. Then the amine of amino acids is transported to α-dicarbonyl via Strecker

action to form α-aminocarbonyls, which are easy to be dehydrated. After dehydration, the hexatomic ring with two unsaturated bonds is oxidized to form pyrazine (Shu 1998). For instance, the 1,4-^{13}C-labeled L-ascorbic acid and L-glutamic acid was used to investigate the formation mechanism of pyrazine. L-Glutamic acid is found to be able to produce ammonia during heated in aqueous solution. What's more, some intermediates, such as acetol, and carbohyl which are released from the L-ascorbic acid, could interact with ammonia to form α-amino carbonyl or hydroxyl. Then the pyrazines with roasted, nutty smells are synthesized. Recent studies had found that the peptides could also leads to the production of pyrazine (Eric et al. 2014). Traditional Strecker degradation includes imine hydrolysis after decarboxylation, but there is no free carboxyl group exists in dipeptides. A hypothesized synthesis mechanism is exploited to explain the formation of pyrazine. The α-dicarbonyl initially reacts with dipeptides to form an imine. The 1,5-hydride shift brings about the enolization of the α-aminoketones, and 4-hydroxy-2-azadiene is produced and followed by deprotonation. The imino moiety in 4-hydroxy-2-azadiene is hydrolysis to generate α-aminoketone, which finally forms the pyrazine (Van et al. 2012). Many factors affecting the Maillard reaction could also influence the formation of pyrazines, such as temperature, heating time, pH, water activity, amino acids or peptides, sugars, and other compounds in foods.

41.3.1 Temperature and Heating Time

The heating temperature is a curial factor in determining the reaction rates and the production of pyrazine (Zhao et al. 2019). It has been verified that almost no pyrazine is detected in the Maillard reaction with glycine at 100 °C; when the temperature increased by 30 °C, pyrazine and its typical derivatives (2,5(6)-dimethylpyrazine and trimethylpyrazine) are founded. In addition, the various kinds of pyrazines are discovered with high content at 150 °C and 180 °C, which indicates a positive correlation between the content of pyrazine and temperature (Van et al. 2012). What's more, the temperature could influence the types and amount of pyrazine through affecting on pH of the reaction system. The initial pH in Maillard reaction containing the L-cysteine and D-xylose was detected at 7.4 at the temperature for 80 °C. After heating at with at 140 °C for 2 h, the pH was decreased to 5.37. The temperature promotes cysteine to accelerate the formation of some acidic compounds (formic and methylglyoxal), which is able to lower the pH (Eric et al. 2014). The heating time often adjusted with temperature during the thermal process, which the time is set for several minutes for high temperature. For instance, the heating time is set as 4 h at a relative low temperature (120 °C), while it is set as 7 min at a high temperature (300 °C).

41.3.2 pH Values

Pyrazines, as the volatiles with special organoleptic properties, are controlled by the pH values at reaction. In this case, it is necessary to evaluate the influence of pH on

pyrazines to control the flavor of thermally treated food. It has been documented that high pH facilitated the generation of pyrazines (Koehler and Odell 1970). A model Maillard reaction at 140 °C for 2 h with different pH 5.0–9.0 was conducted to measure the influence of pH on the amount of pyrazines. In the reaction between L-ascorbic acid and acidic acids (glutamate and aspartate), the content of pyrazines increased with the upward pH value, and it is nearly impossible to detect pyrazines at pHs 5.0–6.0, even it reached maximum at pH 9.0. Also, it is found that the acidic amino acids are much more sensitive to pH value compared with neural acids, which can produce more pyrazines at same pH values. In addition, the impact of pH values on pyrazine generation of basic amino acids is much smaller in comparison with the neutral amino acids, especially the pH at 8.0–9.0. Besides, the basic condition can contribute to the reset and disintegrate of L-ascorbic acid and promote the amino group existed in amino acids to react with the sugar. In general, high pH value almost has no effect on the formation of pyrazines, but has a deep influence on acidic amino acid. The basic group in basic amino acids could contribute to the formation of pyrazines, and the acidic groups could prevent the formation of pyrazines (Tan and Yu 2012; Yu and Zhang 2010a, b).

41.3.3 Moisture Content

The production of pyrazines has a strict requirement on water activity. The presence or absence of water also is a necessary parameter that influences the odor profiles in foods. A Maillard reaction among glucose, glycine, diglycine, and triglycine was carried out at different water content (0%, 10%, 25%, 50%, 75%, and 100%) for 1 h at 160 °C to explore the impact of water content on volatile (pyrroles and pyrazines) generation. It has observed that the substituted pyrazines occupy a large proportion among volatile in all the reaction systems, and their contents increase along with the increased content of water. Because of the limited mobility and solubility, the production of volatile is slightly restrained. The slight restriction may be related with the formation of water during the Maillard process. Besides, the 2-ethyl-5-methyl-, 2-ethyl-3-methyl-, 2-ethenyl-6-methyl-, 3-ethyl-2,5-dimethyl-, and 2-ethyl-3,5-dimethylpyrazines were detected with the water in low and high levels. In addition, water can dominate the degradation of peptides and the reaction rates (Oliyai and Borchardt 1994; Labuza 1980). It has been pointed out that a maximum yield of pyrazines can be obtained when the water activity is 0.33 in a model system. However, a low pyrazine production can be seen when the water activity exceeds 0.33, which may due to the excessive water produced in several condensation processes of Maillard reaction (Eichner and Karel 1972; Scalone et al. 2015).

41.3.4 Free Amino Acids or Peptides

The types of free amino acids can result in the different yields of pyrazines. For instance, the reaction with L-serine can produce more pyrazines compared with

L-threonine, which may be related with the melting point of L-serine (222 °C) is lower than that of L-threonine (256 °C) (Shu 1999). The addition of leucine to the traditional Maillard reaction causes the formation of 3-methylbutylpyrazine, while the addition of valine leads to the synthesis of 2-methylpropyl pyrazines. These results demonstrated that the different types of peptides could determine the formation of different pyrazines. In relation to the peptides in food, oligopeptides were regarded as the vital precursors on promoting the flavor of fried or roasted foods. The polymerization extent and composition of amino acids play an important role in controlling the amount and kinds of pyrazine. The content of pyrazine produced by glycine or triglycine was apparently higher than that produced by diglycine or tetraglycine (Oh et al. 1991). And a high-content pyrazine formed by dipeptides was detected, which was more than that formed by free amino acids and dipeptides, and the latter can generate more pyrazines than tripeptides (Van et al. 2010, 2012). In addition, pyrazines were produced by glucose or glyoxal with different dipeptides which with lysine at N-terminus (Lys-Lys, Lys-Gly, Lys-Ala, Lys-Leu, Lys-Ser, Lys-Glu, Lys-Cys, or Lys-Phe). When acted with glyoxal, small amount of unsubstituted pyrazines is formed compared with the reaction to glucose. The content of pyrazine (2,5(6)-dimethylpyrazine and trimethylpyrazine) in the reaction with Lys-Leu dipeptides is higher than that react with corresponding amino acids, which may be related with the higher $K_{O/W}$ value of amino acids. Interestingly, the unsubstituted pyrazines are produced more when acting with amino acids. And it can be seen that the different reactivity of N-terminal peptide-bound lysine and R- and ε-amino group of free lysine can produce different kinds of pyrazine (Van et al. 2010). Proline, valine, or leucine at the N-terminus leads to a high content of 2,5(6)-dimethylpyrazine and trimethylpyrazine than the glycine, alanine, or serine at the N-terminus, which may due to the similar structure of dipeptides of Val-Gly, Val-Lys, and Leu-Gly. At the same time, compared with other dipeptides, the speed of pyrazines forming in the reaction between methyglyoxal and Val-Gly, Val-Lys, and Leu-Gly is slower, which may be linked with the huge side group of valine and leucine and is prevented by the α-position deprotonation on amide moiety (Van et al. 2010).

41.3.5 Carbonyl Compounds

It has been confirmed that the generation of pyrazines is closely associated with the type of sugar which the product of sugar is carbon source of pyrazines. The reaction systems involved the leucine and various sugars (fructose, glucose, rhamnose, and the mixture of glucose and fructose (3:1)) are conducted at 100 °C with 1 h. The results show that the reaction with fructose has the maximum output (approximately 8500 μg) of pyrazines. Besides, the more various types of pyrazines are generated when applied rhamnose; and the yield of 2-isoamyl-6-methylpyrazine is the most one compared with other sugars (Ara et al. 2017). The reaction with rhamnose is able to produce more pyrazines than high-fructose corn syrup. And rhamnose affects not only the composition of pyrazines but also the output of C1, C2, C3, and C4 pyrazines (Coleman and Steichen 2006).

41.3.6 Others

Several new Amadori-type conjugates produced during the Maillard reaction are regarded as the crucial intermediate products influencing the formation of pyrazines. The Amadori-type conjugates generated from the aminoketones was regarded as the promoter of pyrazine formation, which may be caused by the subsequent splitting reaction (Zou et al. 2018). The reagent ratio also impacts the formation of pyrazines. It has been confirmed that the different ratio of hydrolyzed whey protein and glucose leads to different content of pyrazines. The amount of pyrazines is at a low level when the protein/glucose ratios is 1:0.08 or 1:0.16, which may be due to the low content of dicarbonyl compounds produced by glucose. As the ratio reaches to 1:0.5 and 1:0.67, the high yield of pyrazines can be discovered. However, the production of pyrazines turns to a downward trend with the increase of the ratio. The excessive glucose is able to generate more carboxylic acids (acetic acid) to decrease the pH of the reaction system (Scalone et al. 2015). It is estimated that some representative pyrazines with roasted and nutty flavor are not detected when the pork loin is roasted. They are easy to be discovered under the deep-fried treatment, which indicates that the cooking methods can also determine pyrazines formation (Yang et al. 2017). At the same time, the oil can be oxidized to act with Strecker degradation products of amino acids to influence formation of pyrazines. The olive oil could produce the most content of pyrazines and followed by sunflower and canola oil. Interestingly, the yield of 2-methylpyrazine, 2,5-dimethylpyrazine, and 2,3-dimethylpyrazine are produced more compared with other oil (Whitfield and Mottram 1992), which maybe the rich content of linoleic acid in sunflower oil. The phenolic compounds ((+)-catechin, quercetin, gallic, ferulic, and caffeic acids) added or existed in thermal can determine the production of pyrazines. It is concluded that the phenolic compounds can inhibit the formation of N^{ϵ}-(carboxymethyl) lysine and prevent the generation of pyrazines. Also, the phenolic compounds have different inhibition effect on the N^{ϵ}-(carboxymethyl)lysine formation, which due to their special thermal stability (Mildner-Szkudlarz et al. 2017). It also proposed that the phenolic compounds serve as the reactive radical precursors' scavenger to wipe out some precursors such as dialkylpyrazine and thus prevent the formation of pyrazines (Wang and Ho 2013). With the formation of pyrazines as a pH-dependent reaction, the addition of phenolic acids decreases the pH of system which further decreases the levels of pyrazines (Yu and Zhang 2010b). What's more, the epicatechin is found to be able to inhibit the formation of pyrazines via affecting the activity of C2, C3, and C4 sugar fragments (Troise et al. 2015).

41.4 Enrichment and Detection

The concentration of pyrazines in food is low. It is necessary to establish an efficient enrichment method for the analysis of pyrazines in liquor. In the present, the main four enrichment methods are liquid-liquid extraction (LLE) (Bañeras et al. 2013),

solid-phase microextraction (SPME) (Wu and Xu 2013), supercritical CO_2 extraction (Fan et al. 2007), and ion exchange (Tae et al. 2013), respectively.

41.4.1 Liquid-Liquid Extraction

Liquid-liquid extraction is one of the unit operations for separating homogeneous liquid mixtures. Liquid mixtures can be separated in a certain solvent because of the solubility difference of each component in liquid mixture. Liquid-liquid extraction involves at least three substances including solute ***A*** and original solvent ***B*** in the raw material liquid and extractant ***S***. There are three types of three-component systems formed by the added extractant and raw material liquid (***A*** + ***B***) as follows: (1) solute ***A*** is completely soluble, but the extraction agent ***S*** and the original solvent ***B*** are completely insoluble, forming ***A*** pair of completely insoluble mixed liquid; (2) the extraction agent ***S*** is partially miscible with the original solvent ***B*** and completely miscible with the solute ***A***, forming ***A*** pair of partially miscible mixed liquid; (3) extraction agent ***S*** is partially miscible with not only the original solvent ***B*** but also the solute ***A***, forming two pairs of partially miscible mixed liquid. Liquid-liquid extraction method can remove volatile components of water, ethanol and interference, and is a good method for the determination of pyrazines. Many researchers extracted pyrazines by liquid-liquid extraction. It is existed method that 15 g fermented grains were weighted at first. After grinding, 30 mL 12% (v/v) ethanol was added to soak for 10 min, and then ultrasonic treatment was performed for 30 min. After centrifugation or filtration, 5 mL of supernatant liquid was extracted and put into a 15-mL extraction bottle, 1.5 g NaCl was added, and a manual sample injector with 2 cm-50/30 μm fiber head was inserted, and the sample was extracted at 60 °C for 40 min in headspace. The extraction head was quickly removed and immediately inserted into the GC injection port (temperature at 250 °C). The samples were injected by thermal analysis for 3 min for pyrazine detection and analysis (Beck et al. 2003).

41.4.2 Solid-Phase Microextraction

The principle of SPME is to apply a layer of polymer stationary phase, such as polydimethylsiloxane or polyacrylate, with a thickness of 30~100 μm on a fused silica fiber about 1 cm long. The fiber is connected to a stainless steel plunger shaped like a syringe device and contracts into the stainless steel needle. The plunger contacts fiber from the needle and the sample solution or headspace so that the analyte is adsorbed and distributed to the coating layer. The analyte, concentrated on the fibers, is released into the column by thermal desorption at the GC entry. In the case of HPLC, the analyte adsorbed on the fiber is transferred to the column by virtue of SPME-HPLC interface (Spietelun et al. 2010). SPME is characterized by sampling, extraction, enrichment, and injection. General sample pretreatment methods

can only complete one or two steps, while SPME integrates multiple steps to simplify the sample pretreatment process according to its own characteristics. SPME is easy to operate and directly interacts with the solid-phase coating and hardly consumes solvent. It can reduce the cost and protect the environment. The speed of SPME depends on the time required for analyte distribution equilibrium, which can be reached within 2~30 min. The technique is suitable for the enrichment of trace or trace components. Furthermore, SPME-mass coupled technology was proposed for accurate extraction. For GC/MS instrument, the sample mixture was separated through chromatographic column and then moved into the mass spectrometer in the high vacuum environment. Positively charged fragments are accelerated by electric field into the quality analyzer, which can detect the number of ions with different mass quantity (abundance) mapping, which is the mass spectrogram. If the mass spectrogram of the chromatographic peak location matches the mass spectrogram of the standard compound in the spectrum library with a high matching rate and the same retention time, the chemical structure of the component can be identified. Therefore, SPME-GC-MS is a new extraction separation and identification technology. After the substance is pre-treated by SPME, it is separated in the GC. And then the mass spectrogram is obtained in the MS, which is analyzed by mass spectrogram and combined with the retention time of GC to identify the structural formula of the compound (Vas and Vékey 2004). Compared with traditional methods, this technique has the advantages of no solvent, simple and fast, economic and nontoxic, high accuracy and sensitivity, and convenient combination. In the recent years, many of the pyrazines in liquor are extracted through SPME. For example, researchers take 150 mL wine sample in a beaker and adjust its pH value to 1 with 6 mol/L H_2SO_4. The concentrated liquid was collected, and its volume was recorded by evaporation and concentration at 60 °C for 30 min. The concentration pH was 12 with 6 mol/L NaOH. Then the 50/30 μm DVB/CAR extracted fiber was aged at 250 °C for 30 min at the GC inlet. The prepared liquor sample and standard solution 1 mL were respectively added to the empty top bottle, and then sodium chloride was added to the empty top bottle until the solution was saturated, and a magnet was added to stir the rotor and sealed. The magnetic agitator is stable for 15 min at 2800 r/min 40 °C to reach gas-liquid equilibrium. The aged-extracted fiber is inserted into the headspace of the headspace bottle (the extracted fiber does not touch the solution) and continuously stirred and extracted for 20 min at the same temperature. After extraction, the extracted fiber is pulled into the needle sheath, and the SPME device is removed from the headspace bottle. The SPME device was inserted into the injection port of the GC for sample injection, and it was removed after thermal desorption at 260 °C for 5 min. The conditions of GC were as follows: the injection port temperature 260 °C and the splitless mode for injection. The initial column temperature was 60 °C, and the velocity rose to 260 °C within 10 min at 20 °C/min. The carrier gas was nitrogen, and the constant flow rate was 2 mL/min. The mass spectrum conditions were ionization mode EI, electron energy 70 eV, interface temperature 280 °C, threshold 100, scanning/second 2.67, and scanning mass number 15–600 amub (Lojzova et al. 2009).

41.4.3 Supercritical CO_2 Extraction

Ion exchange refers to the unit operation of mass transfer separation process by means of exchange of ions in a solid ion exchanger with ions in a dilute solution for the purpose of extracting or removing certain ions in the solution. Ion exchange is a reversible equivalent exchange reaction (Kazarian et al. 2013). The ion-exchange resin is sandwiched between the anion exchange membranes to form a single processing unit and constitutes a freshwater chamber. The ion exchange rate decreases as the degree of cross-linking of the resin increases and increases as the particle decreases. Ion exchange is a liquid-solid phase reaction process, which involves the diffusion process of substances in liquid and solid phases. Typical ion exchangers are ion-exchange resins (functionalized porous or gel polymer), zeolites, montmorillonite, clay, and soil humus (Traynor and Ahmad 2018). Ion exchangers are either cation exchangers, which exchange positively charged ions (cations), or anion exchangers, which exchange negatively charged ions (anions). There are also amphoteric exchangers that are able to exchange both cations and anions simultaneously. However, the simultaneous exchange of cations and anions can be more efficiently performed in mixed beds, which contain a mixture of anion- and cation-exchange resins, or pass the treated solution through several different ion-exchange materials. Ion exchange is a reversible process, and the ion exchanger can be regenerated or loaded with desirable ions by washing with an excess of these ions. The method of ion exchange of pyrazine is as follows: take 20 g of resin, and wash it repeatedly with 2 N NaOH, water, 4 N HCI, water, and acetone until the final acetone immersion liquid is colorless. Wet method is loaded into the glass column, washed with 20 ml l N NaOH, and then washed with distilled water until neutral for use. The pH value of samples (50 mL) was adjusted to 12.5 by 2 N NaOH, and then the samples were transferred to the exchange column at a flow rate of 5–10 (mL/min). 25 mL of mixture (2 mL 2 N NaOH mixed with 23 mL of methanol) and 25 mL of water were used to remove impurities. Then it was eluted with 25 mL 4 N HCl and 25 mL of water, collected with 3 × 10 mL CH_2Cl_2 extraction, eluted with 50 mL of acetone-water (4:1, V/V), collected and extracted with 3 × 10 mL CH_2Cl_2 Then combined with the extracts were combined and washed with a 10 mL 2% $NaHCO_3$ solution. The organic acid was removed, dried over anhydrous Na_2SO_4, and concentrated to 0.5 mL. Then, 0.5 mL derivatization reagent and several K_2CO_3 were added to the concentrated liquid. Finally, the mixture was refluxed at 60–70 °C for 2.5 h, dried with N_2, and dissolved with 0.5 mL of n-hexane to carry out gas chromatography (Calull et al. 1992; Nagarale et al. 2006).

41.4.4 Other Methods

Worben et al. (2006) have revealed that vacuum distillation could remove most of the ethanol and water in the wine and then extracted with CH_2Cl_2, GC-MS separation, and identification of pyrazines in rum and whiskey. Lu et al. (1989) have

reported that cationic resin enriched nitrogen compounds in white wine and a total of 12 pyrazines were detected. Yu et al. (1992) found that the acidification-extraction method was conducted to analyze nitrogen-containing compounds in liquor and obtained 36 kinds of nitrogen-containing compounds including 27 pyrazines. Xu et al. (2008) have used the liquid-liquid extraction combined with normal phase chromatography to detect 32 kinds of pyrazine compounds in Jiannanchun wine by full two-dimensional gas chromatography-flying gas chromatography-mass spectrometry (GC×GC-TOF/MS).

41.5 Biological Activities

Pure pyrazines are rarely found in nature and mostly in the form of derivatives. There are many substitute pyrazines in nature. These pyrazines carry substituents on one or more of the four ring carbon atoms. These substituents may include oxygen-containing functional groups, such as alkoxy and acyl groups, or sulfur-containing mercaptan or sulfide groups. Alkylpyrazines contain carbon and hydrogen substituents, and 70 different compounds have been identified such as phenazine, pteridines, and tetramethylpyrazine (Rudolf and Sugima 2010; Wagner et al. 1999). Pyrazine is less basic (pKa = 0.7) than pyridine (pKa = 5.2), pyrimidine (pKa = 1.3), or pyrimidine (pKa = 2.3). Studies have shown that pyrazine derivatives are best absorbed at intestinal pHs 5–7. In humans and laboratory rodents, oral replacement pyrazines are rapidly absorbed and excreted from the gastrointestinal tract, which is possible to ingestion of flavorings containing pyrazine derivatives without adverse health effects. Pyrazine sequences are found in many compounds, which are the source of unique flavors and aromas in several foods and wines (Sumoto et al. 1991; Rohovec et al. 2010). Although pyrazines are widely studied as a flavor component of food, its derivatives are widely used in medicine, food, and chemicals. Pteridines are compounds based on a pyrimido[4,5-b]pyrazine ring system. These compounds exhibit different biological functions and have been widely concerned in the medical field. Pteridines derivatives have been synthesized and determined for bioactivity. They showed the great potential for drug development by targeting a variety of human diseases including cancer, chronic inflammatory diseases, and microbial infections (Kompis et al. 2005).

Cancer is the second leading cause of death in the world. The number of cancer cases is expected to double in the next 20 years (Alan Kambiz and Freidoun 2008). In order to reduce the resistance of cancer drugs to cancer cells, pteridines have been widely studied and shown to have many anticancer effects. Over the past half century, methotrexate (pteridines derivative) has been used as an anticancer drug, either alone or in combination with other chemotherapy drugs. It was used to treat breast cancer, cutaneous T-cell lymphoma, lymphoblastic leukemia, lung cancer, and advanced non-Hodgkin's lymphoma Carmona-Martínez et al. (2018). It has been reported that pteridine-based compounds have a variety of biological activities, such as anti-inflammatory (Hoffmann et al. 2003; Li et al. 2015), analgesic (Gomtsyan et al. 2004), anti-nematode, antibacterial (Shen et al. 2007), and antivirus activities.

Inflammation is an immune process triggered by the presence of pathogens or damaged tissues. In the process of inflammation, the production of signaling molecules such as cytokines and the aggregation of immune cells help to eliminate pathogens and restore tissues. However, most mechanisms designed to kill pathogens and/or kill dead cells also damage normal cells. Nonsteroidal anti-inflammatory drugs are commonly used to reduce inflammation. However, they are not recommended to treat chronic diseases because of their side effects. In recent years, the anti-TNF drugs have been used to treat autoimmune inflammatory diseases. Most patients do not respond to these drugs and can have adverse effects, such as immunosuppression or neurological failure. It is of great significance to develop a new type of anti-inflammatory drug. Currently, 88 different types of pteridine derivatives have been studied for this purpose (Li et al. 2017). Most pteridine derivatives can inhibit TNF-α and IL-6 of pro-inflammatory cytokines in vitro and are treated by lipopolysaccharide in the human macrophage like myeloid leukemia cells of the HL-60 cell model without resulting in cytotoxicity.

The Ca^{2+}-activated K^{+} (BKCa) channel or Maxi K is expressed in smooth muscle cells and is an important regulator of vascular tension. The opening of BKCa channels inhibits voltage-dependent Ca^{2+} channels, restricting extracellular Ca^{2+} access to smooth muscle cells and inhibiting vasoconstriction (Ledoux et al. 2006). The BKCa channel is the target area for relaxation factors released by endothelial cells including nitric oxide (Bolotina et al. 1994) and endothelium-derived hyperpolarizing factor (Archer et al. 2003; Weston et al. 2010). Changes in the expression or BKCa channel activity have been found to be associated with vascular dysfunction in various diseases, such as atherosclerosis, hypertension, diabetes, and metabolic syndrome (Amberg and Santana 2003; Borbouse et al. 2009; Sébastien et al. 2003). Tetramethylpyrazine is an alkaloid compound isolated from *Ligusticum*. Its cardioprotective effects are attributed to vasodilation, anti-inflammatory, antioxidant, and Ca^{2+} homeostasis activities (Guo et al. 2016). In a recent study, tetramethylpyrazine was shown to have strong anti-endoplasmic reticulum stress activity. In endoplasmic reticulum stress coronary endothelial cells, tetramethylpyrazine and endoplasmic reticulum stress inhibitors have the similar inhibitory effects on the activation of unfolded protein response sensor. And this inhibitory effect has a significant protective effect on endothelial function in hypertension-related conditions (Mak et al. 2017; Sun et al. 2019). In addition, tetramethylpyrazine has been used in clinical treatment with mild side effects for ischemic, cerebral infarction and central nervous system degenerative diseases, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis (Tang et al. 2011; Yang and Jiang 2010). Tetramethylpyrazine may significantly prevent lipid peroxidation and necrosis of neurons by eliminating oxygen free radicals (Zhikuan et al. 2008). At present, more and more evidences show that tetramethylpyrazine combined with other treatments can significantly reduce the drug resistance caused by chemotherapy and inhibit the proliferation and metastasis of melanoma cells, lung cancer, gastric cancer, and other cancer cells (Huang et al. 2012; Ji et al. 2014). Interestingly, the recent study showed that tetramethylpyridine significantly protected hippocampal neurons by co-culture of glioma neurons while inhibiting glioma

cell survival in vitro (Fu et al. 2008). It can make glioma subside and prolong the survival of glioma transplanted rats. Tetramethylpyrazine may have the potential to treat glioblastoma (Chen et al. 2013).

Phenazine is a kind of secondary metabolite produced by gram-positive bacteria, gram-negative bacteria or palaeomethane bacteria (Laursen and Nielsen 2004). In addition, it is also a class of nitrogen-containing heterocyclic compounds, which have extensive antibacterial properties. Their properties vary based on the type and location of functional groups (Pierson and Pierson 2010). Natural and synthetic phenazines have many biological activities, such as antihypertensive drugs, antiparasitic drugs, antimalarials, neuroprotectants, free radical scavengers, and anticancer agents (Laursen and Nielsen 2004; Laura et al. 2010; Mavrodi et al. 2006). Krishnaiah et al. (2018) found that 6,9-dichloro-n-(methylsulfonyl)phenazine-1-carboxamide and 9-methyl -*N*-(methylsulfonyl)phenazine-1-carboxamide showed certain effects on drug-resistant *Escherichia coli* and *Staphylococcus aureus*.

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Phenylpropanoids (Phenylpropenes) in Diets

42

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Contents

Abstract

Phenylpropanoids are a large group of phytochemicals that are biosynthesized from the amino acids, phenylalanine, and tyrosine, and they contain at least a phenyl ring linked to a three-carbon alkyl (propyl) group (C_6–C_3 structure). They also form one of the common groups of bioactive phytochemicals that are distributed in plant-based diets. A majority of phenylpropanoids contains

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an unsaturated three-carbon alkenyl chain and is commonly known as phenylpropenes, e.g., cinnamic acid and its derivatives. Thus, the main coverage of this chapter is on phenylpropene derivatives found in food plants and their bioactivities that are established from in vivo testing using either animal models or human trials. The chapter also briefly deals with bioavailability and metabolism of these bioactive dietary phytochemicals.

Keywords

Phenylpropanoids · Phenylpropenes · Phenolics · Bioactivity · Secondary metabolite · Bioavailability · Food source · Plant · Marketed products · Patents

42.1 Introduction

Phenylpropanoids are a large group of phytochemicals that are biosynthesized from the amino acids, phenylalanine, and tyrosine, and they contain at least a phenyl ring linked to a three-carbon alkyl (propyl) group (C_6–C_3 structure). They are the building blocks and/or intermediates for the biosynthesis of more complex natural products, e.g., coumarins, flavonoids, isoflavonoids, and lignans (Sarker et al. 2002; Sarker and Nahar 2007; Koeduka et al. 2014; Atkinson 2016; Marei and Abdelgaleil 2018). These compounds are distributed widely in the plant kingdom but predominantly found in essential oil-producing plants from the families of Apiaceae, Lamiaceae, Lauraceae, Myrtaceae, and Rutaceae, many of those plants are food plants. The vast majority of phytochemicals of this class contain an unsaturated three-carbon alkenyl chain and are commonly known as phenylpropenes, e.g., cinnamic acid and its derivatives; thus, the main coverage of this chapter will be on phenylpropene derivatives. Diversity in the phenylpropene core structure is generally governed by the position of the propenyl double bond position as allyl-phenylpropenes and propenyl-phenylpropenes and various substitutions on the phenyl ring as well as oxidation status on the C_3 side chain, leading to the formation of terminal alcohol, aldehyde, or carboxylic acid (Fig. 1) (Koeduka 2018). Many components of plant essential oils are phenylpropenes, e.g., eugenol, and they often offer distinctive fragrance to the oils. Because of their fragrance, they are used as flavoring agents in processed foodstuffs (Atkinson 2016; Marei and Abdelgaleil 2018). In plants, phenylpropanoids are known

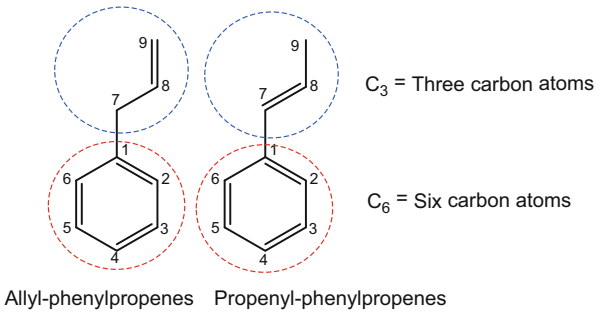

Fig. 1 Core structures of phenylpropenes with different positions of the double bond

to provide protection against biotic and abiotic stresses (Koeduka et al. 2014) and act as chemical defense against various pathogens including microbial attacks (Gang et al. 2002; Özcan and Chalchat 2006; Atkinson 2016).

The tropical and subtropical plants, especially their fruits, contain a high concentration of phenylpropenes, which are biosynthesized through the shikimic acid pathway from their precursor phenylalanine by forming an intermediate cinnamic acid (Fig. 2). Later on, this leads to the biosynthesis of different structural analogs and derivatives incorporating side chain modifications, changes in functional groups, isomeric variation, and oxidation. As these compounds form the major group of phytochemicals within the food plants and often possess culinary significance and medicinal values, they attract food scientists or phytochemists working with food plants for various chemical, pharmacological, and toxicological studies. The traditional medicinal uses of some phenylpropene-containing plants are summarized in Table 1.

42.2 Bioactive Constituents

Aroma of phenylpropenes, especially in edible plants and their folklore medicinal uses, has always intrigued the phytochemists to study their potent bioactive constituents. The amounts of phenylpropenes present in popular culinary species like cloves, fennel, star anise, nutmeg, cinnamon, sweet basil, and rosemary are high. In addition to culinary values, all these plants have significant medicinal value as antimicrobial, antioxidant, anti-inflammatory, anthelmintic, expectorant, and anticancer agents, because of the presence of a variety of phenylpropenes. Notable bioactive phenylpropenes (Fig. 3) isolated from dietary plants include apiole (**1**) from parsley (Parthasarathy et al. 2008), anethole (**2**) from anise (Parthasarathy et al. 2008), caffeic acid (**3**) from coffee (Ross 2005), chavicol (**4**) from allspice (Parthasarathy et al. 2008), cinnamaldehyde (**5**) from cinnamon (Parthasarathy et al. 2008), curcumin (**6**) from turmeric (Parthasarathy et al. 2008), estragole (**7**) from fennel (Gross et al. 2009), eugenol (**8**) from clove (Jirovetz et al. 2006), ferulic acid (**9**) from popcorn (Zhao and Moghadasian 2008), isochavicol (**10**) from apple (Atkinson 2016), isoeugenol (**11**) from apple (Atkinson 2016), methyleugenol (**12**) from apple (Atkinson 2016), methyl isoeugenol (**13**) from strawberry (Atkinson 2016), myristicin (**14**) from nutmeg (Parthasarathy et al. 2008), rosmarinic acid (**15**) from rosemary (Parthasarathy et al. 2008), safrole (**16**) from sassafras (Long et al. 1963), and sinapic acid (**17**) from lemon (Nićiforović and Abramovič 2014).

42.3 Bioavailability and Metabolism

High intake of fruits and vegetables, the key source of phenylpropanoids/phenylpropenes, reduces the chances of degenerative diseases. These dietary sources have different rates of absorption by the systemic circulation followed by the different percentage of bioavailability and metabolism. To make a nutrient bioavailable, it needs to be converted into a bioaccessible chemical from the food matrix so that the gut cells can absorb it. Different phenylpropenes have different rates of

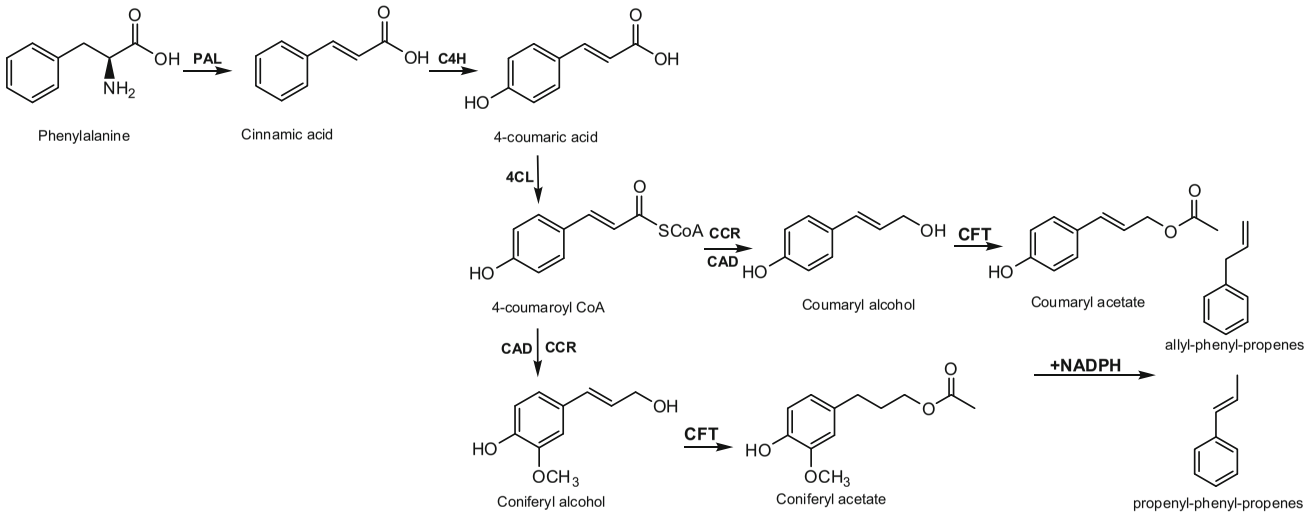

Fig. 2 A generalized representation of phenylpropene biosynthesis (*PAL*, phenylalanine ammonia lyase; *C4H*, cinnamic acid 4-hydroxylase; *4CL*, 4-coumarate:CoA ligase; *CCR*, cinnamoyl-CoA reductase; *CAD*, cinnamyl alcohol dehydrogenase; *CFAT*, coniferyl alcohol acetyltransferase)

Table 1 The traditional medicinal uses of some phenylpropene-containing dietary plants

Plants		Traditional medicinal uses	References
Scientific names	Common names		
Amomum subulatum Roxb.	Large cardamom	To treat inflammation, tuberculosis, malaria, digestive disorders, snake bites	Jafri et al. 2001; Ravindran and Madhusoodanan 2002; Verma et al. 2010
Apium graveolens L.	Celery	Used as a kidney and heart tonic, menstrual stimulant, blood purifier and for the treatment of low blood pressure and bacterial infections	Lans 2006; Mantle and Tiran 2009; Hechtman 2013; Kooti et al. 2015
Artemisia dracunculus L.	Tarragon	Used as a digestive stimulant, sedative, menstruation inducer, analgesic for toothache, antivenom, and antibacterial agent	Hechtman 2013; Chevallier 2016
Cicer arietinum L.	Chickpea	Used as an aphrodisiac and for the treatment of otitis, leprosy, bronchitis	Duke 2002; Bewley et al. 2006
Cinnamomum tamala (Buch.-Ham.) T. Nees & C.H. Eberm.	Bay leaves	Used in anorexia, diarrhea, and spermatorrhea and as a brain tonic	Parthasarathy et al. 2008; Kapoor 2017
Cinnamomum verum J. Presl	Cinnamon	Used to treat asthenia, bronchitis, gout, influenza, leucorrhoea, and diarrhea and as an aphrodisiac and emmenagogue	Duke 2002; Parthasarathy et al. 2008; Price and Price 2012
Citrus limon L.	Lemon	Used to treat hypertension, insomnia, ulcer, boils, stomatitis, and skin infection	Price and Price 2012; Lim 2012a; Baser and Buchbauer 2015
Coffea spp. L.	Coffee	Used as an anti-drowsiness, menstrual remedies, metabolism booster, enema to remove toxins from liver, cholagogue, cardiotonic, and neurotonic, and to treat asthma	Castleman 2001; Ross 2005; Mantle and Tiran 2009
Coriandrum sativum L.	Coriander	Effective against carbuncles, biliousness, catarrh, colic, dyspepsia, halitosis, and bacterial infections and used as an aphrodisiac and labor inducer	Duke 2002; Parthasarathy et al. 2008; Preedy et al. 2011; Baser and Buchbauer 2015
Cucumis melo L.	Melon	Used to treat bronchitis, fever, hepatosis, nephrosis, eczema, anasarca, and liver disorders	Duke 2002; Preedy et al. 2011; Lim 2012b

(continued)

Table 1 (continued)

Plants		Traditional medicinal uses	References
Scientific names	Common names		
Curcuma longa L.	Turmeric	Used as an anthelmintic, blood purifier, depurative, emmenagogue, and hepatoprotective agent and to treat bronchitis, biliousness, leukoderma, rheumatism, and cystitis	Duke 2002; Parthasarathy et al. 2008; Benzie and Wachtel-Galor 2011
Elettaria cardamomum L.	Small cardamom	Remedy for indigestion, nausea, and pulmonary disease and used as an analgesic, abortifacient, diuretic, and anti-aflatoxin	Parthasarathy et al. 2008; Lim 2013a
Foeniculum vulgare Mill.	Fennel	Used as an anti-inflammatory, antispasmatic, emmenagogue, gastroprotective, hypotensive, and bronchodilatory agent	Parthasarathy et al. 2008; Rahimi and Ardekani 2013; Lim 2013b
Fragaria ananassa Duchesne	Strawberry	Well-known as a cardioprotective, antidiabetic, anti-infective, and anti-hypertensive agent	Lim 2012c; Kraft 2016
Glycine max (L.) Merr.	Soybean	Used to treat dysentery, osteoporosis, cardiovascular disease, and postmenopausal complications	Kim et al. 2006; Chen et al. 2012; Kichu et al. 2015
Illicium verum Hook.f.	Star anise	Effective as an antirheumatic, antimicrobial, carminative, emmenagogue, galactogogue, sedative, and stomachic agent	Parthasarathy et al. 2008; Wang et al. 2011; Lim 2013c
Malus domestica Borkh. nom. Illeg.	Apple	Treatment for biliousness, constipation, impotency, and sore spots	Duke 2002; Lim 2012d
Morus atropurpurea Roxb.	Mulberry	Used as an aphrodisiac, expectorant, odontalgic agent, and laxative; also used to treat dysmenorrhea and metrorrhagia	Duke 2002; Lim 2012e; Rahman and Khanom 2013
Musa spp.	Banana	In diarrhea, dysentery, colitis, epilepsy, leprosy, hemorrhoids, and heartburn	Kumar et al. 2012; Lim 2012f
Myristica fragrans Houtt.	Nutmeg	Used as an aphrodisiac, antiemetic, stomachic, carminative, emmenagogue, and abortifacient and to treat halitosis, inflammation, and dyspepsia	Parthasarathy et al. 2008; Lim 2012g; Okonkwo and Ogu 2014

(continued)

Table 1 (continued)

Plants		Traditional medicinal uses	References
Scientific names	Common names		
Myrtus communis L.	Myrtle	To treat urinary infections, vaginal discharge, bronchial congestion, sinusitis, dry cough, epilepsy, hemorrhoids, hay fever, hepatobiliary deficiency, hypothyroidism, prostate congestion, and prostatitis	Lim 2012h; Price and Price 2012
Ocimum basilicum L.	Sweet basil	Used as an antibacterial, antifungal, stimulant, expectorant, menstrual stimulant, alexipharmic, and antipyretic	Mantle and Tiran 2009; Bilal et al. 2012
Origanum vulgare L.	Oregano	Useful in asthenia, respiratory infection, urinary infection, and cold	Price and Price 2012; Guillén et al. 2017
Passiflora edulis Sims.	Passion fruit	Effective as an anthelmintic, antimicrobial, diuretic, and sedative	Dhawan et al. 2004; Lim 2012i
Petroselinum crispum (Mill.) Fuss	Parsley	Used in the treatment of diabetes, skin infections, hyperuricemia, constipation, and induction of abortion. Also used as an antispasmodic, emmenagogue, sedative, carminative, and expectorant	Kuete 2017; Ravindran 2017
Phyllanthus emblica L.	Indian gooseberry	Used in diabetes, anemia, leukorrhea, inflammation, bronchitis, cephalalgia, scurvy, diarrhea, and osteoporosis	Gaire and Subedi 2014; Variya et al. 2016; Charmkar and Singh 2017
Pimenta dioica (L.) Merr.	Allspice	Used as a bactericidal, fungicidal, digestion enhancer, analgesic, and antipyretic agent	Benzie and Wachtel-Galor 2011
Pimpinella anisum L.	Anise	Used as an analgesic, carminative, disinfectant, diuretic, antispasmodic, expectorant, stomachic, and tranquilizer	Shojaii and Abdollahi Fard 2012; Preedy 2015
Piper nigrum L.	Black pepper	Effective as an analgesic, antiseptic, antispasmodic, antitoxic, aphrodisiac, febrifuge, laxative, and stomachic	Ravindran 2000; Parthasarathy et al. 2008; Mantle and Tiran 2009

(continued)

Table 1 (continued)

Plants		Traditional medicinal uses	References
Scientific names	Common names		
Rosmarinus officinalis L.	Rosemary	Used to treat urinary tract infections, nervous disorders, hair loss, enuresis, gout, hay fever, hepatobiliary deficiency, impotence, and tachycardia. It is a traditional astringent, carminative, rubefacient, expectorant, emmenagogue, and diaphoretic	Price and Price 2012
Sassafras albidum (Nutta.) Nees	Sassafras	Well-known blood and kidney cleanser, blood thinner, and antirheumatic	Cavender 2006
Salvia officinalis L.	Sage	Used for the treatment of diabetes, hyperhidrosis, leukorrhea, menopause problems, irregular periods, malignancy, meningitis, neuritis, and vertigo	Price and Price 2012
Solanum lycopersicum L.	Tomato	Remedy for nausea and stomach upset	Cavender 2006
Syzygium aromaticum (L.) Merrill & Perry	Clove	Used as an antispasmodic, analgesic agent, carminative, aphrodisiac, anesthetic, expectorant, and emmenagogue. Also used in hypotension, impotence, influenza, labor problem, malodor, neuralgia, toothache, and tuberculosis	Parthasarathy et al. 2008; Price and Price 2012; Lim 2014
Terminalia chebula Retz.	Myrobalan	Remedy for indigestion, edema, dermatosis, uterine and vaginal disorders, asthma, jaundice, candidiasis, hepatomegaly, vesicular and renal calculi, leprosy, anorexia, and arthritis. Also used as a laxative, astringent, and cardiac tonic	Khare 2004; Ashwini et al. 2011
Trigonella foenum-graecum L.	Fenugreek	Used as a carminative, thermogenic, galactogogue, emollient, and poultice. Also used for bronchitis, menorrhagia, diabetes, dyspepsia, and typhoid	Duke 2002; Parthasarathy et al. 2008

(continued)

Table 1 (continued)

Plants		Traditional medicinal uses	References
Scientific names	Common names		
Vitis vinifera L.	Grapes	Used to treat cachexia, cholera, smallpox, diarrhea, allergies, wounds, and inflammation	Nayak et al. 2010; Mckenna et al. 2011
Zea mays var. *everta*	Popcorn	Used to treat kidney and gall bladder stones, cardiac problems, and tumors	Rahmatullah et al. 2010

bioavailability and metabolism because of their chemical diversity in presence/absence of certain functionalities contributing to solubility and absorption through GI tract.

For this instance, we can take the example of ferulic acid (FA; ferulic acid, **9**), which in comparison to the other dietary phenylpropenes studied so far, is more bioavailable and has antioxidant efficacy. The bioavailability of FA depends on its absorption in the target tissue. According to pharmacological evidences, FA remains in the blood circulation for a longer period and thus resides in the body for a considerable time to get absorbed. Human can ingest around 80–165 mg of FA per meal, which is present in both conjugated and free forms. Around 56.1% of FA rapidly gets absorbed by the enterocytes as conjugative derivatives. Just within 15–30 min of FA ingestion, its absorption rate remains high. About 50% of FA reaches the liver and enters into the hepatocytes and 6% of that secrete in the bile. An amount of 49.9% of the remaining conjugated FA gets distributed into the bloodstream, gastric mucosa, and peripheral tissues. Around 0.5–0.8% FA is excreted through feces, and the remaining is excreted through urine. Mainly the foregut absorbs free FA and feruloyl monomers (e.g., γ-oryzanol), which further metabolized into FA-glucuronides and FA-sulfates by conjugation in the liver and intestinal mucosa. Furthermore, a part of FA undergoes β-oxidation to metabolize in the liver and produces the metabolites dihydroferulic acid and vanillic acid. These metabolites by concatenating with free FA flow through the circulatory system to reach to the target organs like the intestine, spleen, lungs, and kidneys. In the intestine, the esterases present in the lumen hydrolyze the FA to free FA for immediate absorption. Chiefly, the feruloyl monomers and oligomers of FA are hydrolyzed in the small intestine, whereas the feruloyl polysaccharides and feruloyl oligosaccharides need the assistance of intestinal microflora to be hydrolyzed to produce dihydroferulic acid, hydroxyphenylpropionic acid, and phenylpropionic acid (Fig. 4). This is because of the complex structure of feruloyl polysaccharides, which reduces the interaction between the hydrolyzing enzymes, xylanases and ferulate esterases, and FA polymers. All these products after absorption are further metabolized into benzoic acids and hippuric acids in conjugated form in the liver. Upon completion of this cycle, the metabolites are excreted through urine as

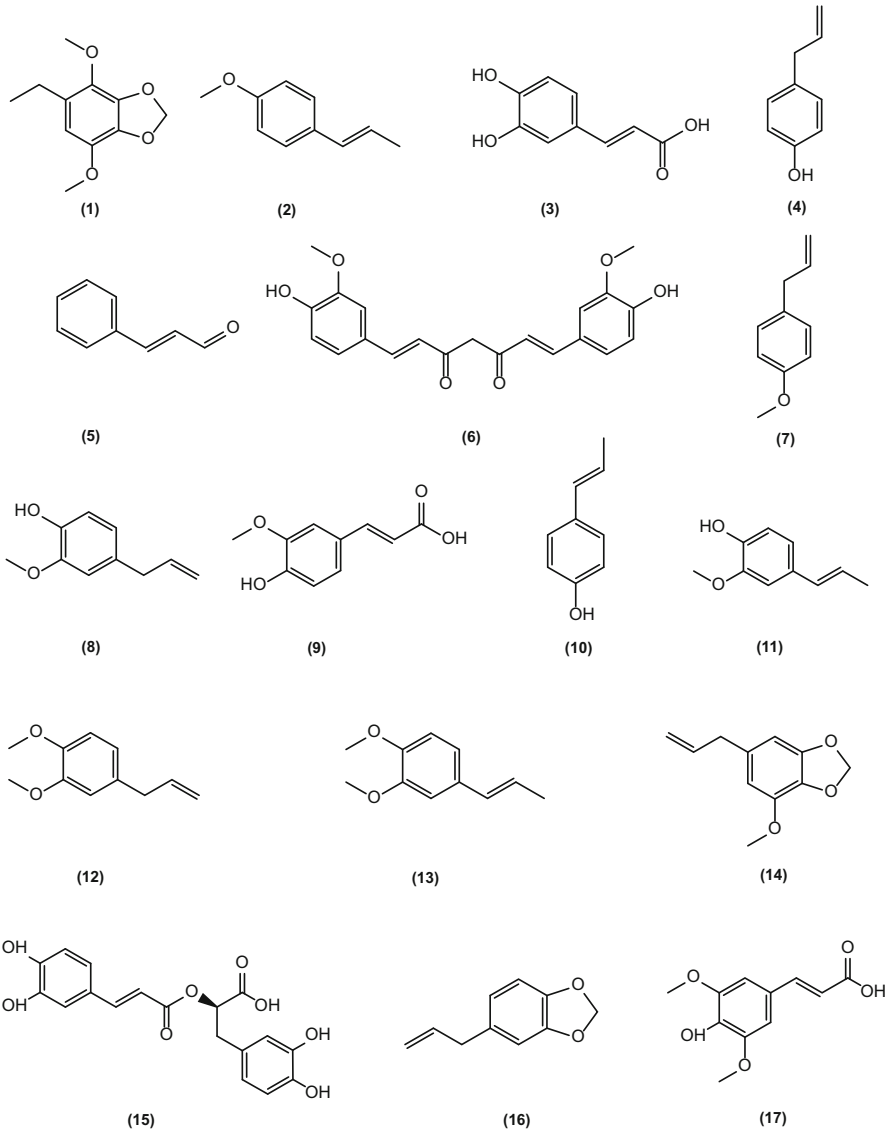

Fig. 3 Bioactive phenylpropenes available in edible plants

3-hydroxyphenyl and 3-methoxy-4-hydroxy phenyl derivatives (Fig. 4). The free or bound forms of dietary FA determine the degree of their absorbability and availability, which has been described in Table 2.

In this process, FA is converted to active form CoA thioester by feruloyl-CoA synthetase, which subsequently is hydrated and catalyzed by β-ketothiolase (Świzdor et al. 2012). The intestinal mucosa and kidney are also involved in this

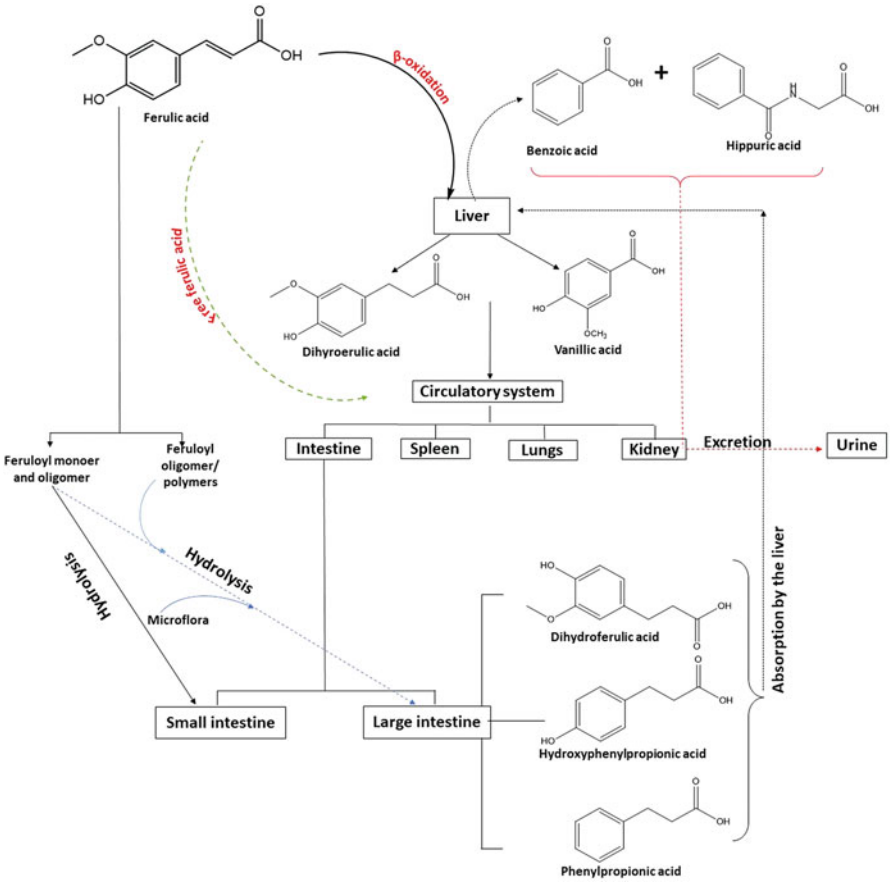

Fig. 4 Diagrammatic representation of ferulic acid metabolism

Table 2 Urinary excretion of dietary FA in human (Zhao and Moghadasian 2008)

Dietary source	Forms of FA	Urinary recovery (%)
Tomatoes	Free and glucuronide FA	25–11
High barn breakfast	Free and conjugated FA	3.13
Pine bark extract	Free and conjugated FA	>92

conjugation process. High intake of FA-rich food (e.g., popcorn) saturates the conjugation enzymes and intensifies the free FA accumulation in plasma and is excreted from the body by a rapid filtration through the kidneys.

Another example can be anethole (**2**), which after oral administration is excreted within 24 h as conjugates of anisic acid and *p*-hydroxybenzoic acid through urine. It follows uniform distribution in the body. Intravenous dose of anethole (**2**) accumulates into the liver, lungs, and brain. It undergoes biotransformation by *N*-oxidation, *O*-demethylation, and epoxidation. The first route is the source of metabolites

like derivatives of cinnamic acid and benzoic acid for ~60–65% dose. The second route is responsible for 40–50% dose. Approximately 30% was excreted as urine metabolites and 0.5% dose excreted as radioactive urea through urine (Tisserand and Young 2014).

Estragole (**7**) is a yet another excellent example. After oral administration of estragole (**7**), it undergoes *O*-methylation and forms chavicol (**4**). It also undergoes oxidation in the allylic side chain and produces the genotoxic metabolites 1′-hydroxyestragole (0.9–8%) and estragole 2′,3′-oxide. 1′-Hydroxyestragole is further converted to its sulfate conjugate 1′-sulfooxyestragole. The liver cells coverts approximately 12.5% of estragole (**7**) into 1′-hydroxyestragole within 24 h and excretes through 0.3–0.4% of ingested estragole (**7**) through urine (Tisserand and Young 2014). Estragole 2′,3′-oxide forms an unstable DNA adduct, which rapidly gets deactivated by detoxification reaction.

Around 75–85% cinnamaldehyde (**5**) is distributed in the gastrointestinal tract, ~2% in blood, ~2% in muscle, ~1% in liver, and ~0.3% in kidneys and other tissues just after 30–60 min of oral administration. It follows the major metabolic pathway of β-oxidation and is excreted through urine in the form of 81.6–84.8% hippuric acid, 3.4–5.1% benzoic acid, and 1.0–1.6% cinnamic acid. The hippuric acid further conjugates with glucuronic acid or glycine (Tisserand and Young 2014). Eugenol (**8**) is rapidly distributed in the blood and kidneys after oral administration. A 70% oral dose is excreted through urine. For an oral dose of 150 mg, 95% is excreted in urine, ~55% as glucuronide and sulfate conjugates and 0.1% as unchanged eugenol (**8**).

42.4 Bioactivities (Animal Aspects)

Phenylpropenes have a wide range of bioactivities like antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, and so on; many of the bioactivities have been documented through in vivo investigations on various animal models. Some notable bioactivities of some frequently used phenylpropenes, which have been tested on animals for the evaluation of their therapeutic efficacy, are summarized in Table 3.

42.5 Benefits (Human Studies)

Phenylpropenes are an essential part of human diet and are ubiquitous in plants. These key plant metabolites are abundantly present in fruits and vegetables. Studies have shown that their potential therapeutic properties play a major role in human health benefits. Some of such vital benefits of phenylpropenes are outlined in Table 4.

Table 3 Bioactivities of some major dietary phenylpropenes, established from animal studies

Phenylpropenes	Bioactivity	Animal model	References
Apiole (**1**)	Anti-inflammatory	Rat	de Cássia da Silveira e Sá et al. 2014
Anethole (**2**)	Anti-inflammatory, convulsive, antioxidant, reproductive toxicity, secrolytic expectorant, sedative activity, antinociceptive, and gastroprotective	Balb/c mice, Swiss mice, mouse, pig, and rat	Freire et al. 2005; Wang et al. 2011; Kang et al. 2013; de Cássia da Silveira e Sá et al. 2014; Marinov and Valcheva-Kuzmanova 2015
Caffeic acid (**3**) and its derivatives	Antioxidant, anti-inflammatory, and antidiabetic	Rat	Zhang et al. 2014
Chavicol (**4**)	Muscle reluctant, anticonvulsant, and gastroprotective	Guinea pig, mice, and rat	Shojaii and Abdollahi Fard 2012
Cinnamaldehyde (**5**)	Anti-inflammatory, anticancer, antidiabetic, and chemoprotective	Balb/c mice, rat, mice	Bandara et al. 2012; de Cássia da Silveira e Sá et al. 2014; Ashakirin et al. 2017
Curcumin (**6**)	Chemoprotective, hepatoprotective, antioxidant, anti-inflammatory, and antifertility	Rats, pigs	Beevers and Huang 2011; Kádasi et al. 2012
Estragole (**7**)	Muscle reluctant, anticonvulsant, analgesic, laxative, and sedative	Rats, mice	Wang et al. 2011; Shojaii and Abdollahi Fard 2012
Eugenol (**8**)	Antioxidant, anti-inflammatory, hepatoprotective, and DNA-protective	Wistar rats, rabbit, rat, bovine, murine	Yogalakshmi et al. 2010; Bachiega et al. 2012; de Cássia da Silveira e Sá et al. 2014
Ferulic acid (**9**)	Antioxidant, anti-inflammatory, antidiabetic, anticholesterolemic, neuroprotective, antithrombosis, and anti-atherosclerosis	Wistar rats, murine, rabbit	Ou and Kwok 2004; Paiva et al. 2013
Isoeugenol (**10**)	Anti-arthritic	Murine	Kaur and Sultana 2012
Methyleugenol (**11**)	Hepatoprotective	F344 rats	Alhusainy et al. 2014
Methyl isoeugenol (**12**)	Anxiolytic and antidepressant	Swiss mice	Fajemiroye et al. 2014
Myristicin (**13**)	Analgesic, anti-inflammatory, antipyretic, antithrombotic, antidiarrheal, and hepatoprotective	Rats, mice	Olajide et al. 2000; Morita et al. 2003

(continued)

Table 3 (continued)

Phenylpropenes	Bioactivity	Animal model	References
Rosmarinic acid (**14**)	Hepatoprotective, anticancerous, and cardioprotective	Sprague-Dawley rat, Balb/c mice, hamster	Nunes et al. 2017
Safrole (**15**)	Anti-inflammatory and anticancer	Balb/c mice	de Cássia da Silveira e Sá et al. 2014
Sinapic acid (**16**)	Anti-inflammatory, anxiolytic, hepatoprotective, cardioprotective, neuroprotective, and antidiabetic	Mice, rats	Nićiforović and Abramovič 2014; Hameed et al. 2016

Table 4 Dietary phenylpropenes and their benefits for human

Therapeutic benefits	Phenylpropenes	References
Acricidal	Anethole (**2**), eugenol (**8**), and isoeugenol (**10**)	Pasay et al. 2010; Koeduka et al. 2014
Anticancer	Cinnamaldehyde (**5**), curcumin (**6**), isochavicol (**10**), rosmarinic acid (**15**), and sinapic acid (**17**)	Beevers and Huang 2011; Al-Dhabi et al. 2014; Nićiforović and Abramovič 2014; Ashakirin et al. 2017
Antidiabetic	Cinnamaldehyde (**5**) and curcumin (**6**)	Beevers and Huang 2011; Bandara et al. 2012
Anti-inflammatory	Anethole (**2**), cinnamaldehyde (**5**), curcumin (**6**), and eugenol (**8**)	Gupta et al. 2013; de Cássia da Silveira e Sá et al. 2014; Marinov and Valcheva-Kuzmanova 2015
Antimicrobial and antiplasmodial	Anethole (**2**), cinnamaldehyde (**5**), eugenol (**8**), ferulic acid (**9**), isochavicol (**10**), isoeugenol (**11**), methyleugenol (**12**), and safrole (**16**)	Miladi et al. 2010; Nazzaro et al. 2013; Diao et al. 2014; Koeduka et al. 2014; Gharib et al. 2017; Marei and Abdelgaleil 2018
Estrogenic activity	Anethole (**2**), eugenol (**8**), isoeugenol (**11**), and methyleugenol (**12**)	Tisserand and Young 2014
Inhibition of lipid peroxidation	Ferulic acid (**9**) and sinapic acid (**17**)	Nićiforović and Abramovič 2014)
Menopausal hot flash reducer	Anethole (**2**), estragole (**7**), and eugenol (**8**)	Shojaii and Abdollahi Fard 2012
Photoprotection of the skin	Caffeic acid (**3**) and ferulic acid (**9**)	Saija et al. 1999; Chan et al. 2004; Lin et al. 2005

42.6 Application in Food (Including Correctly Cooking Foods Rich in Phytochemicals)

The edible plants are rich in phenylpropenes (Table 1) that have great culinary values. This section of the chapter gives a glimpse of the application of these phenylpropene-rich plants in food. The stems, leaves, flowers, and fruits of these plants are widely used as flavoring spices and food additives. For example, sweet basil leaves are extensively used in salads, meats, and soups for its distinct aroma. EOs from allspice is used as a flavoring agent in meat products. The European Commission (2002/113/EC, 2002; 2004/1935/EC, 2004; 89/1107/EEC, 1989) has accepted phenylpropenes like eugenol (**8**) and cinnamaldehyde (**5**) to be used as food flavoring agents. The phenylpropenes in processed foods, like addition of cinnamaldehyde (**5**), enhance the taste of carrot broth. The use of cinnamaldehyde (**5**) in food in vapor form is a potential way. Sometimes the phenylpropenes are used as food preservatives, for example, ferulic acid (**9**). Due to its low toxicity level, it is used as a popular food additive and a natural antioxidant. Rosmarinic acid (**15**) is favored in meat industry due to its meat quality-enhancing capacity. Additionally, it improves the self-life of meat products (Alagawany et al. 2017). Eugenol (**8**) is another extensively used flavoring and fragrance agent. Typically, at a concentration range of 0.05–0.1% (v/v), eugenol (**8**) is used as a food additive. Strawberries treated with eugenol show delayed fruit deterioration.

Additionally, the fruit like bitter gourd rich in phenylpropene is a favorite culinary ingredient in Asian kitchen. People in Taiwan prepare the welcome beverage by mixing its juice with honey. Another example of this category of fruit is carrot. Carrot has worldwide consumer mainly because of its antioxidant values. It can be eaten as steamed, boiled, stews, soups, and salads to get the benefits of the phytochemicals present in it. Juice, cakes, and puddings are also made from carrot to enhance its taste. Apiole (**1**) is abundantly present in parsley leaves, which is used as a freshly chopped form in foods to enhance the flavor. Costmary leaves, having a high content of caffeic (**3**) and ferulic acid (**9**), are used freshly in salads, minced meat, beverages, and cakes to add spicy flavor (Preedy 2015). The cumin seeds are rich in anethole (**2**), estragole (**7**), and ferulic acid (**9**). They have worldwide usage as a spice in various ethnic cuisines for flavoring foods and beverages. The whole seeds are also used as food preservatives to improve the self-life of the food product. Hyssop is another aromatic plant rich in rosmarinic acid (**15**) and caffeic acid (**3**). This plant is famous for its minty aroma and quite popular in Mediterranean kitchen. The phytochemical content is high in its leaves, and therefore they are used in a limited quantity in green salads. The leaves are also used for baking pita breads, which adds the aroma. The whole herbs are used in the form of soup and tea, whereas the young leaves are used as a seasoning for soft cheeses. The nutmeg is the key source of myristicin (**14**), and safrole (**16**) is extensively used in traditional cooking for the sweets preparation. The seed powder is used as a spice to flavor the baked foods, puddings, meats, soft drinks, and vegetables. Its essential oil is used for

flavoring the processed foods. Its mace in dried form is used for flavoring the sweets and dairy products. Another popular phenylpropene-rich culinary ingredient is star anise, containing anethole (**2**). Its essential oil is produced by steam distillation to use in culinary usage. The oil is extensively used as a flavoring agent for alcohols and soft drinks. The aqueous extracts of its fruits are also taken as tea due to its high antioxidant and antimicrobial efficacy. The safrole (**16**)-rich Tasmanian pepper leaf extracts are used in savory dishes. This extract enhances the flavor of cooked meat (Preedy 2015).

42.7 Safety: Toxicity and Side Effects

The human diet frequently includes plants because of their phytochemicals. As these metabolites are produced naturally, therefore they are thought to be safe to consume. Whereas this is not the actual case, in some cases, these naturally occurring substances may lead to severe toxicity and health issues. Phenylpropenes present in fruits and vegetables sometimes may cause toxic effect if they are not handled carefully within the stipulated dose limit. In this section, a few of such kind of examples have been discussed.

One of the commonly available phenylpropene is apiole (**1**). It causes hepatotoxicity and nephrotoxicity and may lead to abortion if the oral consumption limits exceed 53 mg/kg/day. Intake of apiole (**1**)-rich food/fruits/vegetables during pregnancy is not preferable due to high abortifacient activity. The most common apiole (**1**) poisoning are abdominal pain, vaginal bleeding, vomiting, and diarrhea (Tisserand and Young 2014). Hazardous effects of anethole (**2**) overdose are nausea, vomiting, seizures, pulmonary edema, modulation of reproductive hormones, induction of carcinogenicity, and inhibition of blood coagulation if the oral intake exceeds the limit of 54 mg/kg/day. Precautions should be taken while taking the anethole (**2**)-rich content for a person on diabetes or anticoagulant medication because anethole (**2**) interferes with blood glucose and blood clotting cascades. However, anethole (**2**) as a flavoring agent does not have any safety concern (Tisserand and Young 2014; Marinov and Valcheva-Kuzmanova 2015). As a side effect, cinnamaldehyde (**5**) causes skin sensitization and depletes hepatic glutathione. Patients on antidiabetic drugs are advised to avoid cinnamaldehyde (**5**) content because of its antiplatelet aggregation activity. As per the safety concern, estragole (**7**) is a potential carcinogenic and reproductive hormone modulator. It interferes with diabetic medications as it inhibits platelet aggregation. High exposure of eugenol (**8**) and methyleugenol (**12**) potentially becomes carcinogenic. Its maximum oral dose concentration is 233 mg/kg/day, and for dermal usage, the maximum is 0.02%. As a side effect, it causes skin sensitization, whereas isoeugenol (**11**) is corrosive to tissues and hepatocarcinogenic. In general, myristicin (**14**) does not cause any toxicity or side effects, but it is a monoamine oxidase inhibitor (MAOI) and cytochrome P450 (CYP) enzyme inducer. Therefore, patients on MAOIs or

pethidine drugs should avoid myristicin (**14**). High intake of myristicin (**14**) content may cause neurotoxicity. The maximum exposure level recommended for safrole (**16**) is 0.01%; its high dose causes central nervous system depression.

42.8 Marketed Products

Emerging evidences of abundant use of phenylpropenes as essential oils, perfumes, food additives, and flavoring agents have led to manufacture of various marketed products from them. Some of the products are listed below (Table 5) (Source TOXNET).

42.9 Patents

A patent is one of the intellectual rights, where a government authority or license confers a right or title for a certain period of time and excludes any other from making, using, or selling that particular invention under the mentioned time period. The intellectual property of the inventor for methodologies of phytochemical extractions, their application to obtain functional foods, their biological activities, and their application in disease prevention can be protected by the patent right. Researchers working with phenylpropanoids have produced a number of new inventions with these compounds. Some of them are presented in Table 6.

Table 5 Usage of phenylpropenes in marketed products

Phenylpropenes	Marketed products
Anethole (**2**)	Pharmaceutical aid, flavoring cattle feed, and carminative medicine
Cinnamaldehyde (**5**)	Flavor and perfume industry, rubber reinforcing agent, brightener, pesticide, metal coating, corn rootworm attractant, dog and cat repellent, and soil casing mushroom
Eugenol (**8**)	Insecticide, perfumes, essential oils, analgesic medicine, denaturant for alcohol, zinc oxide-eugenol cements for dentistry, meat preservatives, and manufacture of vanillin
Ferulic acid (**9**)	Food preservatives and food supplements for bodybuilding athletes
Methyleugenol (**12**)	Flavoring agent, insect attractant, and oriental fruit fly attractant
Myristicin (**14**)	Psychopharmacological drugs, food flavoring agents, and insecticides
Safrole (**16**)	In manufacture of piperonyl butoxide, as preservatives in mucilage and library paste, perfumes, denaturing fats in soap, and in manufacture of heliotropin

Table 6 Patents on phenylpropene-related research. (Source PubChem)

Phenylpropenes	PubChem CID	Total number of patents
Apiole (**1**)	10659	109
Anethole (**2**)	637563	4987
Caffeic acid (**3**)	689043	1001
Chavicol (**4**)	68148	741
Cinnamaldehyde (**5**)	637511	2579
Curcumin (**6**)	969516	2556
Estragole (**7**)	8815	381
Eugenol (**8**)	3314	3052
Ferulic acid (**9**)	445858	1384
Isochavicol (**10**)	5474441	19
Isoeugenol (**11**)	853433	11
Methyleugenol (**12**)	7127	404
Methyl isoeugenol (**13**)	637776	24
Myristicin (**14**)	4276	87
Rosmarinic acid (**15**)	5281792	22
Safrole (**16**)	5144	972
Sinapic acid (**17**)	637775	525

42.10 Perspectives

Phenylpropenes are detected in a wide range of edible plants, fruits, and vegetables, but the reports related to economically and commercially important plant materials are fewer in number. Most of the studies on phenylpropenes/phenylpropanoids have been conducted in animals, whereas they have a huge impact on human health also. Studies related to human aspects are yet to meet the epitome. Like the protective effect of eugenol (**8**) in strawberry, the other phenylpropenes are in the queue to get such validations. The biosynthesis and pharamcokinetics of all the available phenylpropenes are in need for much deeper understanding to elucidate their role in human health benefits.

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Polyynes in Food

43

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Abstract

So far, more than 1000 polyynes have been identified from nature. Nevertheless, little is known about their function and mechanism in food plants from nutraceutical and/or pharmaceutical perspectives. In this chapter, recent studies on the structure and function of bioactive polyynes in food plants are summarized. In particular, their mechanism, biosynthesis, bioavailability, metabolism, human benefits, safety, patent, and potential applications are discussed whenever the information is available. The information provided here highlights the possible usefulness of the polyynes in food plants for human and animal health, and offers insights into their future research directions.

Keywords

Polyynes · Food · Bioactivity

43.1 Introduction

Plants are a cornerstone of foods, drinks, and medicines that promote human health (Bartolome et al. 2013). The use of plants and their compounds in traditional/folkloric medicine and as functional foods is still quite prevalent in developing countries. Furthermore, plants and their compounds are an extraordinary source of novel drug which leads for different categories of illnesses. Currently, half of all prescribed medicines are developed from terrestrial plants and microorganisms (Newman and Cragg 2016).

Polyynes represent a group of organic compounds with two or more carbon-carbon triple bonds. Naturally occurring polyynes have been isolated from a variety

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of plant species, fungi, bacteria, and invertebrates (Shi Shun and Tykwinski 2006). Since the isolation of the first polyyne from an *Artemisia* plant in 1826, over 1000 polyynes have been identified from nature, mainly from plants. Despite much progress in the identification of polyynes and their derivatives from plants over the past two centuries, information about the function and mechanism of the polyynes in food plants is still fragmented and needs to be updated (Christensen and Brandt 2006; Dembitsky 2006; Negri 2015; Shi Shun and Tykwinski 2006). Extensive pharmacological screening and functional studies have demonstrated that food plant extracts and polyynes display broad biological activities, including anti-metabolic, anticancer, antimicrobial, anti-inflammatory, immunomodulatory, and other activities (Dembitsky 2006). Therefore, they are of great interest, especially in the nutraceutical and/or pharmaceutical industries. However, their safety is poorly studied. In fact, two polyyne-type drugs, neocarzinostatin, and calicheamicin/esperamicin, derived from bacterial compounds, were approved as prescription drugs for cancer therapy despite of their high toxicity (Van Lanen and Shen 2008).

The present chapter focuses on recent studies on the structure and function of bioactive polyynes in food plants. In addition, their mechanism, biosynthesis, bioavailability, metabolism, human benefits, safety, patent, and potential applications will be emphasized whenever the information is available. The information provided here highlights the possible usefulness of the polyynes in food plants and offers insights into their future research directions. The present chapter focuses on recent studies on the structure and function of bioactive polyynes in plants used as foods as examples. Their mechanisms of action, biosynthesis, bioavailability, metabolism, human benefits, safety, patent, and potential applications are outlined whenever the relevant information is available. The information provided here highlights the possible usefulness of the polyynes in food plants and offers insights into future research directions.

43.2 Bioactive Constituents (with Chemical Structures of the Main Bioactive Compounds)

So far, polyynes have been identified from 24 families of higher plants. Among them, six families, Asteraceae, Campanulaceae, Apiaceae, Araliaceae, Torriceliaceae, and Annonaceae, seemed to regularly produce various polyynes (Negri 2015). Vegetable plants (Fig. 1) such as carrot (*Daucus carota*, Apiaceae), celery (*Apium graveolens*, Apiaceae), lettuce (*Lactuca sativa*, Asteraceae), parsley (*Petroselinum crispum*, Apiaceae), parsnip (*Pastinaca sativa*, Apiaceae), fennel (*Foeniculum vulgare*, Apiaceae), aubergine (*Solanum melongena*, Solanaceae), artichoke (*Helianthus tuberosus*, Asteraceae), tomato (*Lycopersicon esculentum*, Solanaceae), caraway (*Carum carvi*, Apiaceae), and *Bidens pilosa* (Asteraceae) are common food plants known to produce polyynes (Bartolome et al. 2013; Crozier et al. 2013). In this chapter, we revisit and discuss the structure and bioactivity of polyynes in the plants of the Asteraceae, Campanulaceae, Apiaceae, Araliaceae, Torriceliaceae, and Annonaceae families. Ninety-four bioactive compounds and their related bioactivities and plant sources are listed in Table 1.

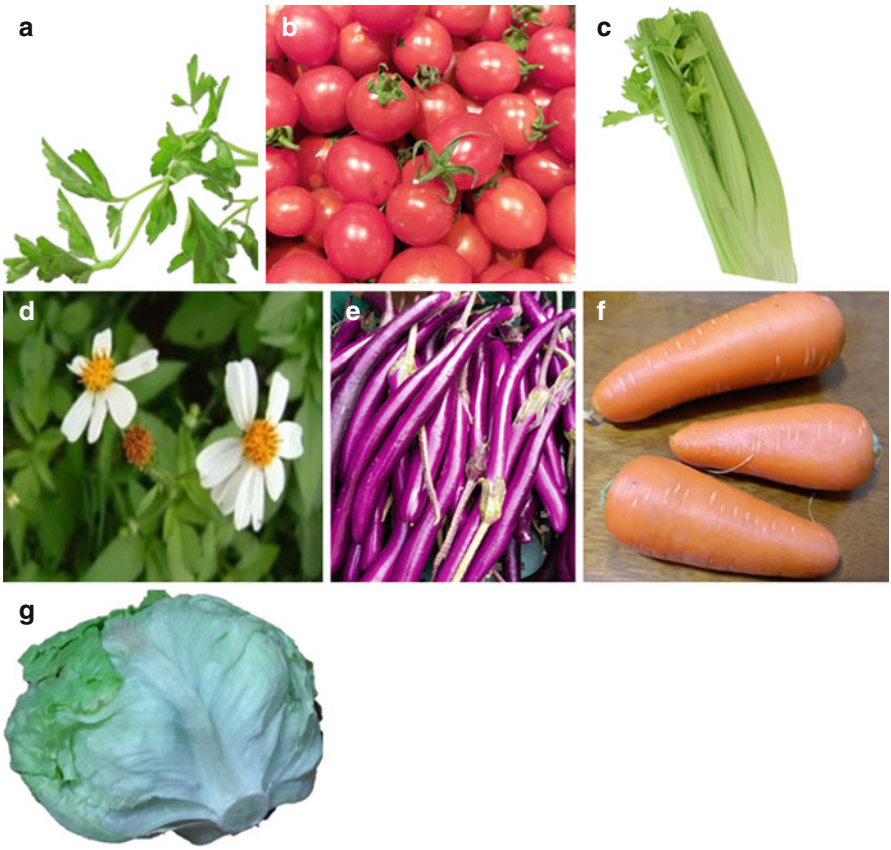
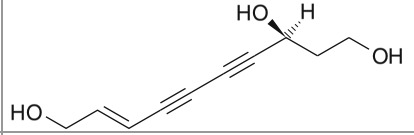
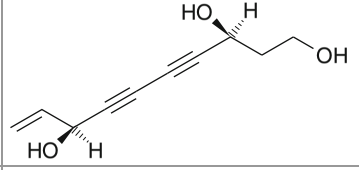
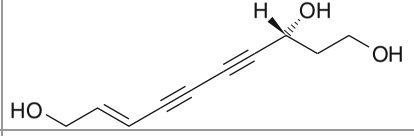
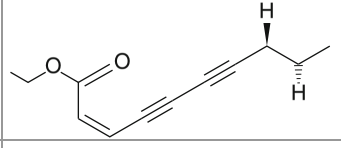
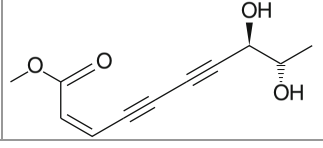


Fig. 1 Some vegetables rich in polyynes: parsley (**a**), tomato (**b**), celery (**c**), *B. pilosa* (**d**), aubergine (**e**), carrot (**f**), and lettuce (**g**)

43.3 Biosynthesis, Bioavailability, and Metabolism

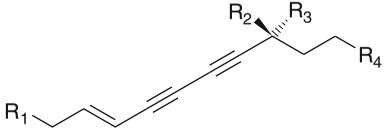
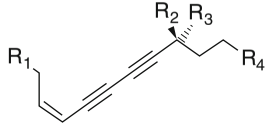
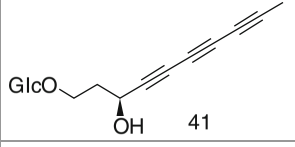
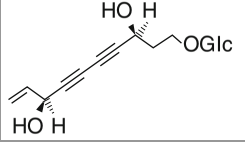
Although the biosynthesis of polyynes in plants and other organisms is not well defined, they are thought to be derived from desaturation and acetylation of fatty acids (Fig. 2) in plants (Yang 2014). Taking *B. pilosa* as an example, polyynes can be found in its various parts including leaves, stems, flowers, and roots (Yang 2014). Polyyne aglycones are normally lipid soluble and absorbed within 30 min via the guts of mice. However, polyyne aglycones are sometimes cytotoxic compared with other phytochemicals such as flavonoids, lignans, terpenoids, coumarins, etc. In addition, polyynes can conjugate with glucose to form their glycosides, which can increase their water solubility, reduce their cytotoxicity, and augment their stability and/or bioactivities. Polyyne glycosides may be degraded by intestinal microbiota in animal guts. Moreover, polyynes are said to be unstable compounds because of their

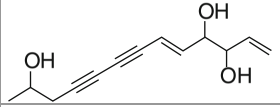
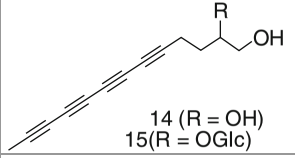
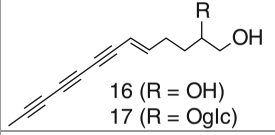
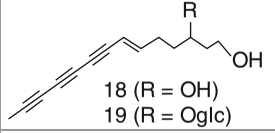
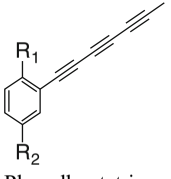
Table 1 Chemical structure, bioactivity, and source of polyynes

S.N.	Name	Biological activities	Plant source	Reference
1		Antipyrectic, anti-inflammatory, antirheumatic	*B. parviflora* (Asteraceae)	(Li et al. 2008)
2		Hepatoprotective, choleretic	*A. capillaris* (Asteraceae)	(Zhao et al. 2014)
3		Hepatoprotective, choleretic	*A. capillaris* (Asteraceae)	(Zhao et al. 2014)
4		Anticancer	*C. albida* (Asteraceae)	(Fukuyama et al. 2012)
5		Antibacterial	*C. canadensis* (Asteraceae)	(Xie et al. 2007)

(continued)

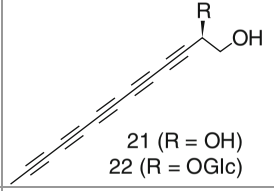
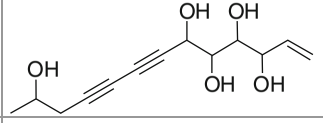
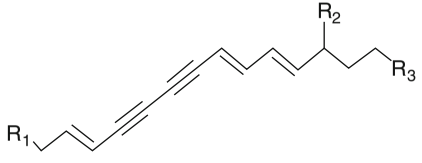
Table 1 (continued)

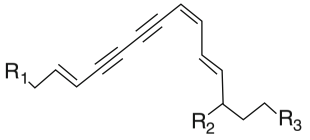
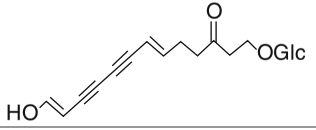
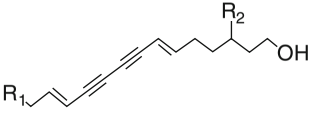
S.N.	Name	Biological activities	Plant source	Reference
6–8	 6 ($R_1 = R_2 = OH$, $R_3 = H$, $R_4 = OGlc$) 7 ($R_1 = OH$, $R_2 = R_3 = H$, $R_4 = OGlc$) 8 ($R_1 = R_3 = H$, $R_2 = OH$, $R_4 = OGlc$)	NO inhibition Antihistamine	*B. bipinnata* (Asteraceae), *B. parviflora* (Asteraceae)	(Li et al. 2004; Wang et al. 2001).
9–10	 9 ($R_1 = R_2 = R_3 = H$, $R_4 = OGlc$) 10 ($R_1 = R_3 = H$, $R_2 = OH$, $R_4 = OGlc$)	NO inhibition Antihistamine	*B. bipinnata* (Asteraceae), *B. parviflora* (Asteraceae)	(Li et al. 2004; Wang et al. 2001).
11	 41	NO inhibition Antihistamine	*B. bipinnata* (Asteraceae), *B. parviflora* (Asteraceae)	(Li et al. 2004; Wang et al. 2001).
12		LOX inhibition	*A. capillaris* (Asteraceae)	(Stavri et al. 2005)

13		Anticancer	*B. pilosa* (Asteraceae)	(Chiang et al. 2007; Wang et al. 2010; Wu et al. 2007b, 2010)
14, 15	 14 (R = OH) 15(R = OGlc)	Anticancer, antidiabetic, antiangiogenic, macrophage activation	*B. pilosa* (Asteraceae)	(Chang et al. 2007, 2013b; Chung et al. 2016)
16, 17	 16 (R = OH) 17 (R = Oglc)	Anticancer, antidiabetic, antiangiogenic,	*B. pilosa* (Asteraceae)	(Chang et al. 2001; Chiang et al. 2007; Sundararajan et al. 2006; Wu et al. 2004, 2007a)
18, 19	 18 (R = OH) 19 (R = Oglc)	Anticancer, antidiabetic, antiangiogenic,	*B. pilosa* (Asteraceae)	(Chang et al. 2001; Chiang et al. 2007; Sundararajan et al. 2006; Wu et al. 2004, 2007a)
20	 Phenylheptatriyne	Antimalarial	*B. pilosa* (Asteraceae)	(Kumari et al. 2009)

(continued)

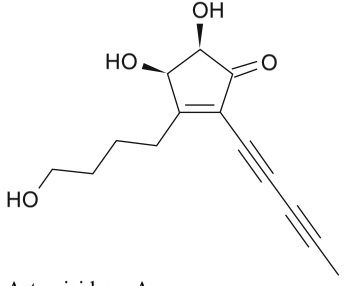
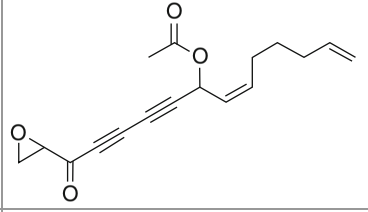
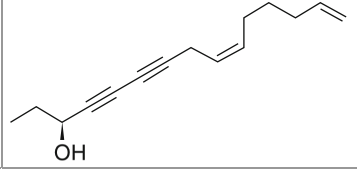
Table 1 (continued)

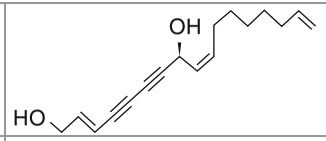
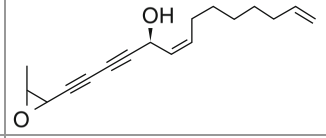
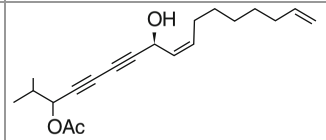
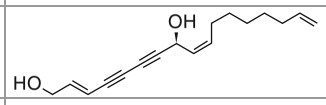
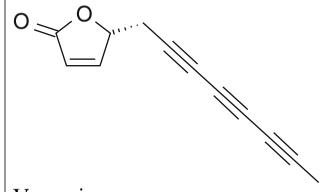
S.N.	Name	Biological activities	Plant source	Reference
21, 22	 21 (R = OH) 22 (R = OGlc)	Antibacterial, antimalarial	*B. pilosa* (Asteraceae)	(Tobinaga et al. 2009)
23		Insecticidal, cytotoxic, antifungal		(Tobinaga et al. 2009)
24–26	 24 (R_1 = OH, R_2 = H, R_3 = $OCOCH_2CH(CH_3)_2$) 25 (R_1 = OH, R_2 = $OCOCH(CH_3)_2$, R_3 = OAc) 26 (R_1 = R_3 = OAc, R_2 = $OCOCH{=}C(CH_3)_2$)	NO inhibition, anti-inflammatory	*A. macrocephala* (Asteraceae)	(Yao and Yang 2014)

27–29	 27 (R_1 = OH, R_2 = H, R_3 = OCOCH = $C(CH_3)_2$) 28 (R_1 = OH, R_2 = $OCOCH(CH_3)_2$, R_3 = OAc) 29 (R_1 = R_3 = OAc, R_2 = $OCOCH(CH_3)CH_2CH_3$)	NO inhibition, anti-inflammatory	*A. macrocephala* (Asteraceae)	(Yao and Yang 2014)
30		Anti-inflammatory	*B. bipinnata* (Asteraceae)	(Wang et al. 2014)
31-33	 31 (R_1 = H, R_2 = OGlc-(2-1)-Ara) 32 (R_1 = OH, R_2 = OGlc-(2-1)-Ara) 33 (R_1 = OAc, R_2 = OGlc-(2-1)-Ara)	COX-2 inhibition	*C. tinctoria* (Asteraceae)	(Zhang et al. 2013)

(continued)

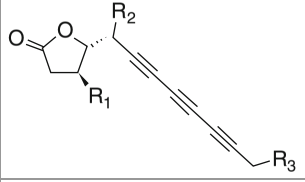
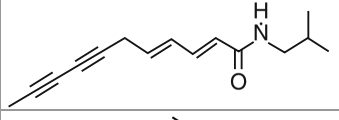
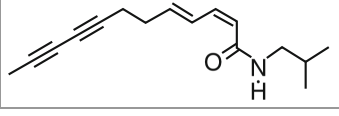
Table 1 (continued)

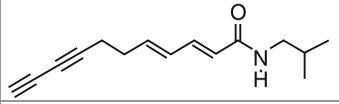
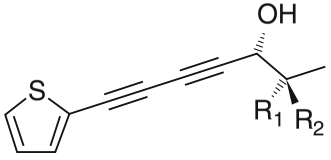
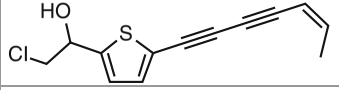
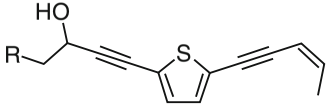
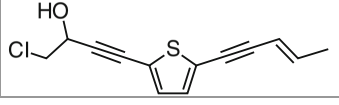
S.N.	Name	Biological activities	Plant source	Reference
34	 Artemisidyne A	Anticancer	*A. lactiflora* (Asteraceae)	(Xiao et al. 2014)
35		Antibacterial	*H. annuus* (Asteraceae)	(Seshimoto et al. 2011)
36		Antibacterial	*H. annuus* (Asteraceae)	(Seshimoto et al. 2011)

37		Anticancer	*G. koraiensis* (Asteraceae)	(Jung et al. 2002)
38		Anticancer	*G. koraiensis* (Asteraceae)	(Jung et al. 2002)
39		Anticancer	*G. koraiensis* (Asteraceae)	(Jung et al. 2002)
40		Anticancer	*G. koraiensis* (Asteraceae)	(Jung et al. 2002)
41	 Vernoniyne	Anticancer Antiviral	*V. scorpioides* (Asteraceae)	(Buskuhl et al. 2009; Klein et al. 2013; Pollo et al. 2013)

(continued)

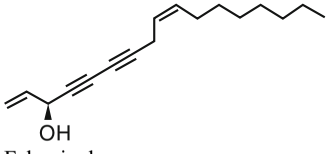
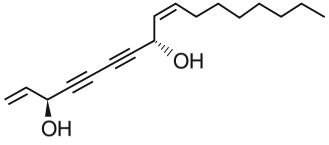
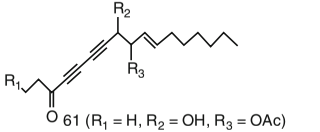
Table 1 (continued)

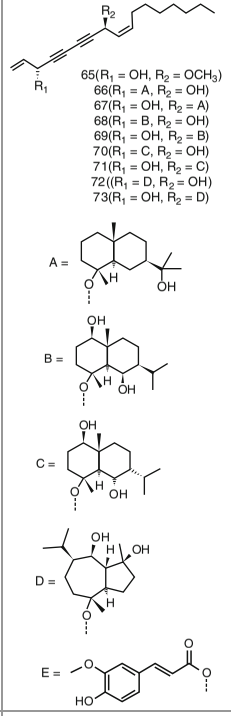
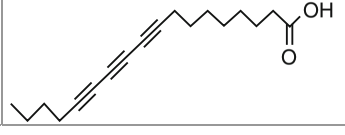
S.N.	Name	Biological activities	Plant source	Reference
42-48		Anticancer Antiviral	*V. scorpioides* (Asteraceae)	(Buskuhl et al. 2009; Klein et al. 2013; Pollo et al. 2013)
49	42 (R_1 = R_2 = H, R_3 = OH) 43 (R_1 = H, R_2 = R_3 = OH) 44 (R_1 = OH, R_2 = R_3 = H) 45 (R_1 = R_3 = OH, R_2 = H) 46 (R_1 = R_2 = H, R_3 = OGlc) 47 (R_1 = OH, R_2 = H, R_3 = OGlc) 48 (R_1 = OGlc, R_2 = H, R_3 = OH)	Antibacterial, antifungal	*M. aurea* (Asteraceae)	(Ahmed and Elela 1999).
50		Insecticidal	*A. dracunculus* (Asteraceae)	(Saadali et al. 2001)
51		Anti-inflammatory	*E. atrorubens* (Asteraceae), *E. purpura* (Asteraceae), *E. pallida* (Asteraceae)	(Schmiech et al. 2009)

52		Anti-inflammatory	*E. atrorubens* (Asteraceae), *E. purpura* (Asteraceae), *E. pallida* (Asteraceae)	(Schmiech et al. 2009)
53, 54	 53(R_1 = OH, R_2 = H) 54(R_1 = H, R_2 = OH)	Antibacterial	*X. subacaulis* (Asteraceae)	(Zhang et al. 2014)
55		Antibacterial	*X. subacaulis* (Asteraceae)	(Zhang et al. 2014)
56, 57	 56(R = Cl) 57(R = OH)	Antibacterial	*X. subacaulis* (Asteraceae)	(Zhang et al. 2014)
58		Antibacterial	*X. subacaulis* (Asteraceae)	(Zhang et al. 2014)

(continued)

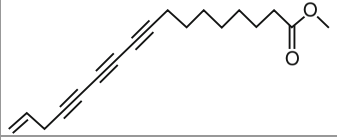
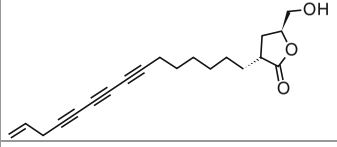
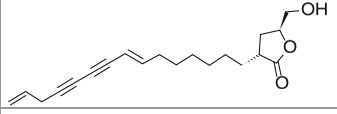
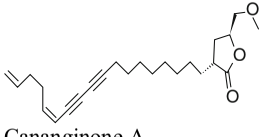
Table 1 (continued)

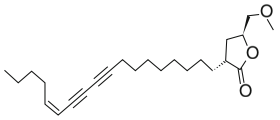
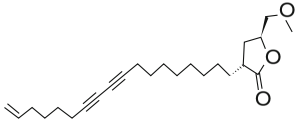
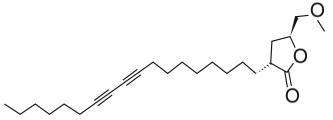
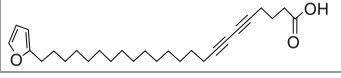
S.N.	Name	Biological activities	Plant source	Reference
59	 Falcarinol	Anticancer	*Daucus carota* (Apiaceae)	(Lund 1992).
60	 Falcarindiol	Anticancer	*Daucus carota* (Apiaceae)	(Lund 1992).
61-64	 61 (R_1 = H, R_2 = OH, R_3 = OAc) 62 (R_1 = H, R_2 = OAc, R_3 = OH) 63 (R_1 = OCH_3, R_2 = OH, R_3 = OAc) 64 (R_1 = OCH_3, R_2 = OAc, R_3 = OH)	Anticancer	*Daucus carota* (Apiaceae)	(Lund 1992).

65-73		Anticancer	*A. graveolens* (Apiaceae)	(Liu et al. 2014; Zidorn et al. 2005)
74		Antimalarial, anti-mycobacterial	*P. cerasoides* (Annonaceae)	(Kanokmedhakul et al. 2007)

(continued)

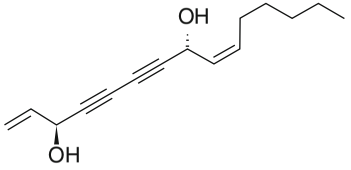
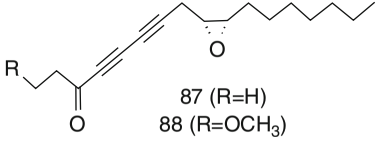
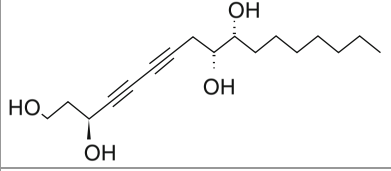
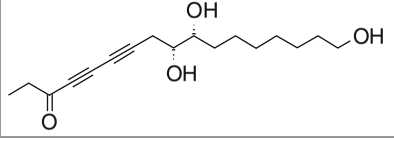
Table 1 (continued)

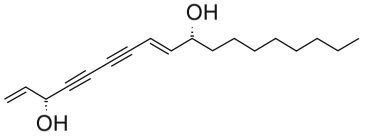
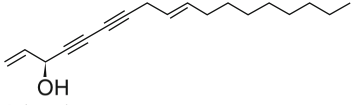
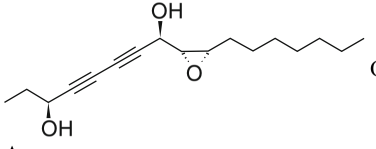
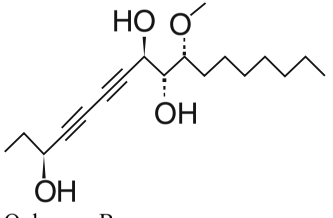
S.N.	Name	Biological activities	Plant source	Reference
75	 Mkiluaynoic acid A	Antifungal		(Li et al. 2009)
76		Anticancer, antifungal, antimicrobial	*M. glabra* (Annonaceae), *M. maingayi* (Annonaceae)	(Zhang et al. 2010)
77		Anticancer, antifungal, antimicrobial	*M. glabra* (Annonaceae), *M. maingayi* (Annonaceae)	(Zhang et al. 2010)
78		Anticancer, antifungal, antimicrobial	*M. glabra* (Annonaceae), *M. maingayi* (Annonaceae)	(Zhang et al. 2010)
79-82	 Cananginone A	Anticancer	*C. latifolia* (Annonaceae)	(Wongsa et al. 2011)

	 Cananginone B  Cananginone C  Cananginone D			
83		Antimalarial, anti-herpes	*P. evecta* (Annonaceae)	(Kanokmedhakul et al. 2006)
84		Anti-HIV	*P. suberosa* (Annonaceae)	(Kanokmedhakul et al. 2006)
85		Anti-HIV	*P. suberosa* (Annonaceae)	(Kanokmedhakul et al. 2006)

(continued)

Table 1 (continued)

S.N.	Name	Biological activities	Plant source	Reference
86	 Araliadiol	Anticancer	*A. cordata* (Araliaceae)	(Cheng et al. 2011)
87–88	 87 (R=H) 88 ($R=OCH_3$)	Inhibition of DAGAT and ACAT	*P.ginseng* (Araliaceae)	(Lee et al. 2004)
89		Antibacterial, antifungal	*P.ginseng* (Araliaceae)	(Fukuyama et al. 2012)
90		Antibacterial, antifungal	*P.ginseng* (Araliaceae)	(Fukuyama et al. 2012)

91	 Stipudiol	Anticancer	*P.quinquefolius* (Araliaceae)	(Liang et al. 2011)
92	 Stipuol	Anticancer	*P.quinquefolius* (Araliaceae)	(Liang et al. 2011)
93	 Oploxyne A	NOX inhibition	*P.quinquefolius* (Araliaceae)	(Yang et al. 2010)
94	 Oploxyne B	NOX inhibition	*P.quinquefolius* (Araliaceae)	(Yang et al. 2010)

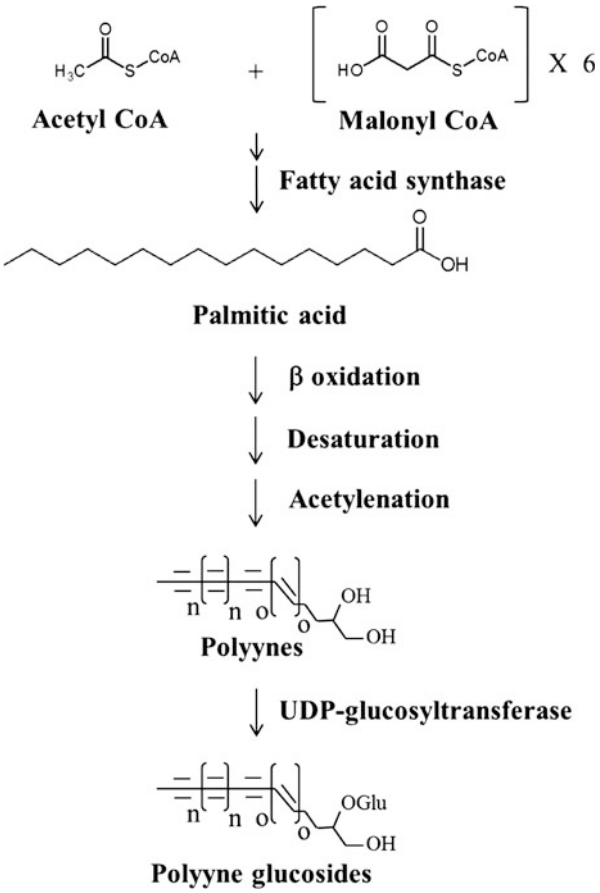


Fig. 2 A putative biosynthetic pathway of polyynes and their glucosides in food plants

carbon-carbon triple bonds. However, the content of polyynes in the Asteraceae plants is high compared to other type of compounds. For instance, the content of *B. pilosa* (Asteraceae) is as high as 0.1–0.3‰ of the dried plant weight (Yang 2014). One argument for their high content in these plants is that they are stable in a milieu of other phytochemicals with high antioxidant activities.

43.4 Bioactivities (in Animals and Humans)

As summarized in Table 1, polyynes from plants used as foods have a variety of bioactivities, such as anticancer, antimetabolic (anti-obese and antidiabetic), antimicrobial (antiviral, antibacterial, antiprotozoan, etc.), anti-inflammatory, immunomodulatory, complement inhibition, diuretic, choleretic, and other activities. Below is an outline of the major bioactivities of the plant extracts and their active polyynes.

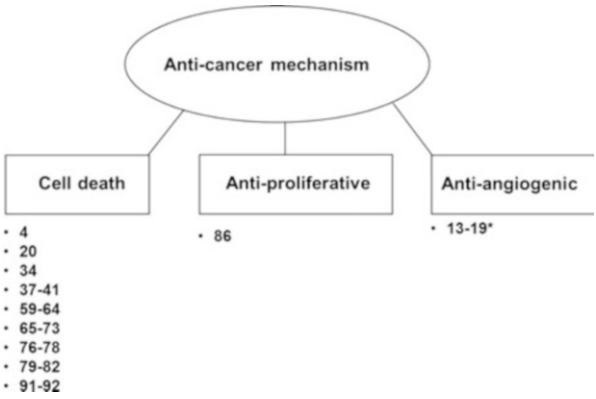


Fig. 3 The underlying mechanisms of the polyynes against cancer. Different polyynes suppress cancer growth via inhibition of cell death, cell proliferation, and angiogenesis. Asterisks (∗) indicate that compounds 13–19∗ promote cell death and inhibit angiogenesis

43.4.1 Anticancer Activity

Plants are an extraordinary source of anticancer drug discovery (Newman and Cragg 2007, 2016; Newman et al. 2003). Over the past 25 years, over 70% of anticancer drugs have been developed from phytochemicals and their derivatives, such as vinblastine, camptothecin, etoposide, paclitaxel, etc. (Newman and Cragg 2016). As delineated in Table 1, the majority of polyynes from edible plants show anticancer properties, i.e., have cytotoxic, antiproliferative, and antiangiogenic bioactivities (Fig. 3). As listed in Table 1, the Asteraceae plants are extremely rich in polyynes. For example, compound **4** isolated from *Conyza albida* (Asteraceae) exhibited cytotoxicity to epithelial (KB) cancer cells (Fukuyama et al. 2012). Compounds **13–19** were initially identified from the edible plant *B. pilosa* (Asteraceae) and identified to have cytotoxic activity against leukemia and carcinoma (Chang et al. 2001; Chiang et al. 2007; Sundararajan et al. 2006; Wu et al. 2004). Moreover, compound **14** promoted apoptosis of endothelial cells and, in turn, suppressed angiogenesis (Wu et al. 2004, 2007a). Further, the antitumor effect and mechanism of compound **14** were able to induce cell apoptosis of cancer cells via induction of caspases 3, 6, and 7 (Kuo et al. 2017). Similarly, phenylhepatriyne (**20**), isolated from *B. pilosa*, was effective against colon and liver cancer cells (Kumari et al. 2009). From the aerial parts of *Artemisia lactiflora* (Asteraceae), Arytemisidyne A (**34**) was identified and shown to be cytotoxic against different cancer cells (Xiao et al. 2014). From the root of *Gymnaster koraiensis* (Asteraceae), compounds **37–40** displayed cytotoxicity against L1210 leukemia cells (Jung et al. 2002). Vernoniyne (**41**) was purified from the leaves and flowers of *Vernonia scorpioides* (Asteraceae). This compound was reported to suppress cancer growth in vitro and in a mouse model of cancer via regulation of caspase activation (Buskuhl et al. 2009). The Apiaceae plants also synthesize polyynes. Vegetables of this family regularly produce common polyynes (Crozier et al. 2013; Lund 1992). Furthermore, related polyynes such as falcarinol (**59**), falcarindiol (**60**), and derivatives (**61–64**) were found in the root of carrot, *D. carota* (Apiaceae). Falcarindiol and its derivatives (**59–64**) were shown to inhibit cancer in vitro and in vivo (Lund 1992). The 8-methyl

ether derivative of falcarindiol (**65–73**) was extracted from the stem of celery, *Apium graveolens* (Apiaceae), and displayed cytotoxicity against leukemia, lymphoma, and myeloma comparable to that of falcarinol and falcarindiol (Zidorn et al. 2005). Other derivatives of falcarindiol, notoethers A-H (**65–73**) of falcarindiol, were identified from the rhizomes and roots of *Notopterygium incisium* (Apiaceae), a Chinese medicinal herb used to treat infection and inflammatory diseases (Liu et al. 2014). Compounds **76–78** from the stem bark of *Mitrephora maingayi* (Apiaceae) were shown to have cytotoxic activity against epithelial, breast, lung, and astrocytoma cancer cells (Zhang et al. 2010). Canangionones A-D (**79–82**) were isolated from the stem bark of *Cananga latifolia* (Annonaceae), a Thai herbal medicine for nasal polyposis and fever (Wongsa et al. 2011). The Araliaceae plants are also rich in polyynes. Araladiol (**86**) was isolated from the leaves of *Aralia cordata* (Araliaceae), a plant that is used as food plant in Asian countries (Cheng et al. 2011). This compound suppressed cell growth of breast cancer cells via inhibition of the cell cycle involving down-modulation of CDK4 and cyclin D and up-modulation of p21 in a p53-independent fashion. In addition, stipudiol (**91**) and stipuol (**92**) were isolated from the rhizomes of *P. stipueantus*, an herb traditionally used in China as a tonic and to treat muscle pain, bleeding, and bruises (Liang et al. 2011).

43.4.2 Antimetabolic Activity

Metabolic syndrome, as known as insulin-resistance syndrome or American syndrome, is a clustering of at least three of the following five medical conditions: central obesity, high blood pressure, high blood sugar, high serum triglycerides, and low serum high-density lipoprotein. Metabolic syndrome increases the risk of heart disease, stroke, and diabetes in humans.

Plants are an extraordinary source of antidiabetic agents. Over 1200 plant species have been claimed to treat diabetes (Habeck 2003; Marles and Farnsworth 1995). One such plant, *B. pilosa* has been shown to treat diabetes (Lin et al. 1994; Marles and Farnsworth 1995; Ubillas et al. 2000), obesity (Liang et al. 2016), and hypertension (Bilanda et al. 2017; Dimo et al. 2002). One seminal study by Ubillas et al. showed that the aqueous ethanol extract of the aerial part of *B. pilosa* at 1 g/kg BW lowered blood glucose in db/db mice, a T2D mouse model (Ubillas et al. 2000). They also used a bioactivity-guided identification strategy to identify two polyynes, compounds **17** and **19**. Moreover, the mixture of the compounds (**17**:**19**) in a 2:3 ratio significantly reduced the blood glucose level and food intake on the second day when administered at doses of 0.25 g/kg twice a day to C5BL/Ks-db/db mice. When evaluated at 0.5 g/kg, a more substantial decrease in blood glucose level as well as more severe anorexia (food intake reduced from 5.8 g/mouse/day to 2.5 g/mouse/day) was observed (Ubillas et al. 2000). This study suggested that compounds **17** and **19** were active ingredients in *B. pilosa* for diabetes (Ubillas et al. 2000). The antidiabetic effect of both polyynes was partially caused by the hunger suppressing effect. However, the anoxic effect of the ethanol extract of *B. pilosa* was not observed in the studies described below. In another study (Hsu et al. 2009), water extracts of *B. pilosa* (BPWE) were tested in diabetic db/db mice, aged

6–8 weeks, with post-meal blood glucose levels of 350–400 mg/dL. Like oral antidiabetic glimepiride, which stimulates insulin release, one single dose of BPWE reduced blood glucose levels from 374 to 144 mg/dL. The anti-hyperglycemic effect of BPWE was relevant to an increase in serum insulin levels, implying that BPWE drops blood glucose concentration through an upregulation of insulin production. However, BPWE showed different insulin secretion kinetics from glimepiride (Hsu et al. 2009). One drawback of current antidiabetic agents is their decreasing efficacy over time. Therefore, the authors investigated the long-term antidiabetic effect of BPWE in db/db mice. BPWE lowered blood glucose, boosted blood insulin, improved glucose tolerance, and reduced the percentage of glycosylated hemoglobulin (HbA1c). Both one-time and long-term experiments strongly support the superior action of BPWE on diabetes (Hsu et al. 2009). Unlike glimepiride which failed to preserve pancreatic islets, BPWE significantly protected against islet atrophy in mouse pancreas. The group also evaluated the antidiabetic effect of three *B. pilosa* varieties, *B. pilosa* var. *radiate* (BPR), *B. pilosa* var. *pilosa* (BPP), and *B. pilosa* var. *minor* (BPM) in db/db mice (Chien et al. 2009). A single oral dose (10, 50, and 250 mg/kg BW) of BPR, BPP, or BPM water extract decreased postprandial blood glucose levels in db/db mice for up to 4 h, and this reduction was dose-dependent. Of note, BPR extract resulted in a higher reduction in blood glucose levels when administered at the same dose as the other two varieties. Further, the BPR extract increased serum insulin levels in db/db mice to a greater extent than the other varieties at the same dose, 50 mg/kg. Three polyynes, cytopiloyne (**15**) and its related polyynes (**17** and **19**), were identified from the three *Bidens* strains though their contents varied. Compound **15** at 0.5 mg/kg exerted better stimulation for insulin production in db/db mice than compounds **17** and **19**. On the contrary, 28-day treatment with the *Bidens* extracts and the three polyynes was then conducted on diabetic mice with postprandial glucose levels from 370 to 420 mg/dL, and glimepiride was used as a positive control. The applied dosages ranged from 10 mg/kg to 250 mg/kg BW. Results showed that the positive control as well as the crude extracts of the three varieties lowered the blood glucose levels in db/db mice. However, only BPR extract, containing a higher content of cytopiloyne (**15**), reduced blood glucose levels and augmented blood insulin levels more than BPP and BPM. The percentage of glycosylated hemoglobin A1c (HbA1c), a long-term indicator of blood homeostasis, was also monitored. HbA1c in the blood of 10- to 12-week-old diabetic mice was 7.9 $\pm$ 0.5%. However, treatment with BPR crude extract (50 mg/kg), glimepiride (1 mg/kg), and compound **15** (0.5 mg/kg) led to HbA1c of 6.6 $\pm$ 0.2%, 6.1 $\pm$ 0.3%, and 6.2 $\pm$ 0.3%, respectively, in the blood of age-matched mice (Chien et al. 2009). Since cytopiloyne (**15**) was the most effective polyyne found in *B. pilosa*, against T2D, it was used for further study of the antidiabetic action and mechanism in another study (Chang et al. 2013b). The data confirmed that cytopiloyne reduced post-meal blood glucose levels, increased blood insulin, improved glucose tolerance, suppressed HbA1c level, and protected pancreatic islets in db/db mice. Nevertheless, cytopiloyne never managed to decrease blood glucose in streptozocin (STZ)-treated mice whose β cells were already destroyed. In addition, cytopiloyne dose-dependently promoted insulin secretion and

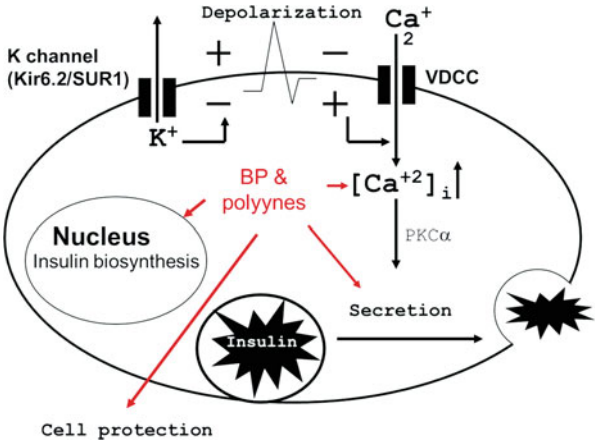


Fig. 4 The mechanism underlying the action of the crude extract of *B. pilosa* and its polyynes (compounds **15**, **17,** and **19**) in T2D. BP and/or its polyynes can treat T2D development via control of β cell function in db/db mice. Their antidiabetic actions are through upregulation of insulin expression/secretion and protection of β cells involving secondary messengers (calcium and diacylglycerol) and their downstream PKCα pathway

expression in β cells as well as calcium influx, diacylglycerol, and activation of protein kinase Cα. Taken together, these mechanistic studies suggest that cytopiloyne acts on T2D via regulation of β cell function (insulin production and β cell preservation) involving the calcium/diacylglycerol/PKCα cascade (Fig. 4). The above studies point to the conclusion that cytopiloyne (**15**) and related polyynes (compounds **17** and **19**) are antidiabetic agents in animal models. The data revealed a new biological action of the polyynes. Similarly, compounds **87** and **88** were isolated from *P. ginseng*, and both inhibited the activity of diacylglycerol acyltransferase (DAGAT) (Lee et al. 2004).

We, for the first time, reported that a standard diet containing 0.5% *B. pilosa* extract dose-dependently decreased fat content, adipocyte size, body weight, and increased protein content in ICR mice and ob/ob mice (Liang et al. 2016). Further, mechanistic studies showed that *B. pilosa* inhibited the expression of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) in adipose tissue (Liang et al. 2016). Finally, we examined the effect of cytopiloyne on adipogenesis in adipocytes. We found that *B. pilosa* significantly decreased the adipogenesis and lipid accumulation. This decrease was associated with the down-regulation of expression of PPARγ and C/EBPα and adipocyte Protein 2 (aP2) and adiponectin (Liang et al. 2016). The data suggest that *B. pilosa* and cytopiloyne suppressed adipogenesis and lipid content in adipocytes and/or animals via the down-regulation of the PPARγ and C/EBPα pathways. Mechanistic studies indicated that compound **15** inhibited the adipogenesis in pre-adipose cells (Liang et al. 2016).

Dimo et al. first stated that the methanol and dichloromethane extract of *B. pilosa* leaves at 75 md/kg/day or more reduced blood pressure in fructose-treated rats (Dimo et al. 2002). Further, the same group confirmed that the ethyl acetate extract of *B. pilosa* leaves at 75 md/kg/day or more reduced blood pressure in L-N^G-nitroarginine methyl ester-treated rats (Bilanda et al. 2017). They suggested that *B. pilosa* exerted its hypotensive action via a reduction of NO synthesis (Bilanda et al. 2017). However, the hypotensive compounds of *B. pilosa* need to be characterized.

43.4.3 Antimicrobial Activity

Polyynes have been shown to be effective against viruses, bacteria, and fungi. Compound **5** was identified from *Conyza canadensis* (Asteraceae). This compound exhibited antibacterial activity (Xie et al. 2007). Compounds **14**, **15**, **20**, **21**, and **22** were isolated from *B. pilosa*. Some polyynes show antibacterial and antimalarial activities (Chiang et al. 2007; Chung et al. 2016; Wang et al. 2010; Wu et al. 2007b, 2010). Compounds **35** and **36** were isolated from *Helianthus annuus*; both exhibited antibacterial activity (Seshimoto et al. 2011). Vernoniyne (**41**) and compounds **42–48** were purified from the leaves and flowers of *Vernonia scorpioides* (Asteraceae). These compounds showed antiviral activity (Buskuhl et al. 2009; Klein et al. 2013; Pollo et al. 2013). Compound **49** was isolated from the aerial parts of *Matricaria aurea* (Asteraceae). This compound exhibited inhibitory activity against bacteria and fungi (Ahmed and Elela 1999). Phenylhepatriyne (**20**), isolated from *B. pilosa*, had antimalarial activity (Kumari et al. 2009). Compounds **53–58** were isolated from *Xanthopappus subacaulis* (Asteraceae), used as a Chinese herbal medicine to treat hematemesis, gastroenteral ulcers, and idiopathic thrombocytopenic purpura. These compounds had antibacterial activities (Zhang et al. 2014). Compound **74** from the extract of the roots of *Polyalthia cerasoides* (Annonaceae), which is used as a tonic or febrifuge in China, was shown to have antimalarial and anti-mycobacterial activities (Kanokmedhakul et al. 2007). Mkiluaynoic acid A (**75**) was isolated from the fruits and stem bark of *Mkilua fragrans* (Annonaceae). Mkiluaynoic acid A had antifungal activity against *Candida albicans* (Li et al. 2009). In addition, compounds **76–78** displayed antimicrobial and antifungal activities (Li et al. 2009). Compound **83** was obtained from the roots of *Polyalthia evecta* (Annonaceae) and is used as a galactagogue in Thailand. This compound showed inhibitory activity against malaria and herpes virus (Kanokmedhakul et al. 2006). Compounds **84** and **85** were purified from *P. suberosa*; both showed anti-HIV activity (Tuchinda et al. 2001). In addition, two polyynes (**224** and **228**) from *P. ginseng* had antibacterial and antifungal activities (Fukuyama et al. 2012).

43.4.4 Anti-inflammatory Activity

Inflammation has four signs, redness, heat, swelling, and pain. Phytochemicals including the polyynes and their derivatives are usually assessed for their anti-inflammatory properties. Compound **1** was identified from the hydroalcoholic extract of *B. parviflora* (Asteraceae) and was found to have antipyretic, anti-inflammatory, and anti-rheumatic activities (Li et al. 2008). Compounds **2** and **3** were isolated from the ethanolic extract of the aerial parts of *A. capillaris* (Asteraceae), a Chinese herbal medicine used for anti-inflammatory, hepatoprotective, diuretic, and choleretic purposes (Zhao et al. 2014). Compound **12** was shown to inhibit the activity of lipoxygenase (LOX), an enzyme involved in inflammatory diseases (Stavri et al. 2005). Compounds **24–26** were identified from the cyclohexane extract of the rhizomes of *Atractylodes macrocephala* (Asteraceae), traditionally used to treat splenic asthenia, edema, anorexia, and

hyperhidrosis in Northeastern Asia (Yao and Yang 2014). These compounds were reported to reduce nitrogen oxide (NO) production and inflammatory mediators (Yao and Yang 2014). Compound **30** was identified from the extract of *B. bipinnata* (Asteraceae), and this compound had anti-inflammatory activity (Wang et al. 2014). Compounds **31–33** were obtained from the capitula of *Coreopsis tinctoria* (Asteraceae). All three compounds inhibited the activity of cyclooxygenase-2 (COX-2) in mouse peritoneal macrophage-stimulated lipopolysaccharide (LPS) (Zhang et al. 2013). Compound **50** was isolated from the extract of *Artemisa dracunculus* (Asteraceae). This compound had insecticidal activity (Saadali et al. 2001). Compounds **51–52** were obtained from the roots of *Echinacea atrorubens* and *E. pallida* (Asteraceae). Compound 132 displayed inhibition of macrophage activation stimulated by LPS (Schmiech et al. 2009). Oploxyne A (**93**) and B (**94**) were identified from the stem of *Oplopanax elatus* (Araliaceae), a medicinal medicine for analgesic and anti-inflammatory purposes (Yang et al. 2010).

43.4.5 Immunomodulatory Activity

The immune system is a host defense system that is instrumental in the maintenance of human health. Imbalance in the immune system can result in infection, autoimmunity, inflammatory diseases, and cancer. For instance, T1D is caused by the autoimmune destruction of pancreatic β cells, leading to insulin deficiency, hyperglycemia, and complications. Immunotherapy is a common approach to treat T1D (Chang et al. 2013a).

Plants and their compounds are commonly used to enhance and/or suppress host immunity in an attempt to treat disorders of the immune system. Plants from the genus *Bidens* were reported to treat 41 categories of diseases such as immune regulation (Bartolome et al. 2013). Compounds **6–8** were obtained from the ethanolic extract of the aerial parts of *B. bipinnata* (Li et al. 2004; Wang et al. 2001). All three compounds indicated anti-allergic activity via inhibition of NO and histamine production in mouse macrophages stimulated by LPS and interferon-γ (Wang et al. 2001). In addition, we reported that *B. pilosa* extract and its butanol fraction decreased Th1 cells and cytokines and increased Th2 cells and cytokines (Chang et al. 2004). This study indicated that the IC_{50} value of the butanol fraction was 200 μg/mL. This inhibition was reported to be partially attributed to cytotoxicity because the butanol fraction at 180 μg/mL could cause 50% death of Th1 cells. Using a bioactivity-directed isolation and identification approach (Fig. 5), three active polyynes, compounds **15**, **17,** and **19,** were isolated from the butanol extract using high pressure liquid chromatography (HPLC) and were then structurally identified by nuclear magnetic resonance (NMR) (Chang et al. 2004; Chiang et al. 2007). All the three polyynes had similar effects on Th cell differentiation to the *B. pilosa* butanol fraction. Moreover, compound **15** showed greater activity than compounds **17** and **19** in terms of enhancement (by 277% compared to 34% and 8%) of differentiation of Th0 to Th2 at 10 μg/mL and inhibition (by 60% compared to 17% and 9%) of differentiation to Th1 at the same concentration (Table 2) (Chang et al. 2004; Chiang et al. 2007).

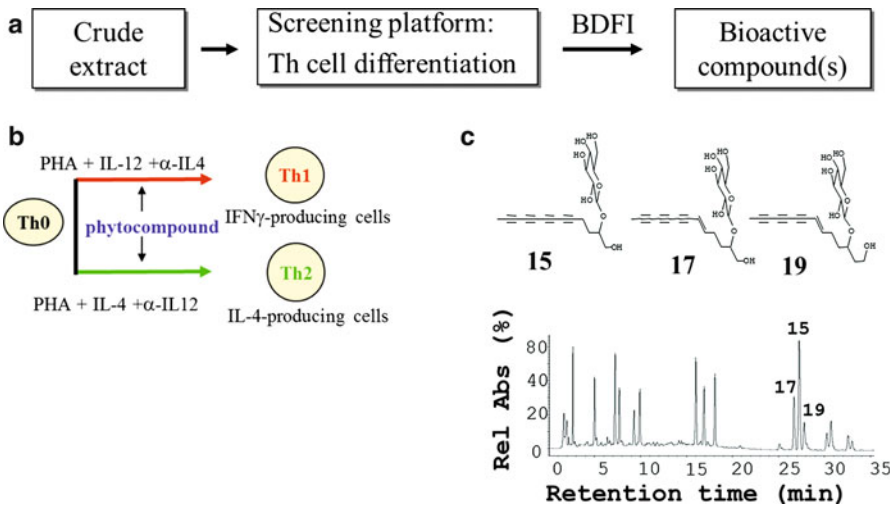


Fig. 5 Bioactivity-directed fractionation and isolation approach to identify three active polyynes that regulate Th cell differentiation. A flowchart of the bioactivity-directed fractionation and isolation (BDFI) strategy describes the use of the screening platform, Th cell differentiation assays to determine bioactive compounds from the crude extract and fractions of *B. pilosa* (A). Bioassays composed of human naïve helper T cells (Th0) can differentiate into type 1 helper T (Th1) cells and type 2 helper T (Th2) cells in the presence of PHA plus IL-12 and anti-IL-4 antibody and PHA with IL-4 and anti-IL-12 antibody, respectively. The crude extract, fractions, and compounds of *B. pilosa* are added to differentiating cells to test the Th cell differentiation (B). Compounds **15**, **17,** and **19** are active compounds that promote Th2 cell differentiation but inhibit Th1 cell differentiation

Table 2 Th1 inhibition and Th2 promotion by the extract (150 μg/ml) and polyynes (10 μg/ml) of *B. pilosa*

	Butanol extract	Compound **15**	Compound **17**	Compound **19**
Reduction of Th1 (%)	32%	75%	9%	17%
Increase of Th2 (%)	103%	277%	6%	31%

The butanol fraction of *B. pilosa* effectively prevented T1D in nonobese diabetic (NOD) mice (Chang et al. 2004). Consistently, this prevention involved down-regulation of Th1 cells or upregulation of Th2 cells. This was proven by intraperitoneal injection of the butanol fraction at a dose of 3 mg/kg body weight (BW), 3 times a week, to NOD mice from 4 to 27 weeks (Chang et al. 2004). This dosage resulted in lower incidence of diabetes (33%). At a dose of 10 mg/kg, the butanol fraction of *B. pilosa* totally stopped (0%) the initiation of the disease (Chang et al. 2004). Th1 cytokine IFNγ and Th2 cytokine IL-4 favor the production of IgG2a and IgE, respectively. To further confirm whether this butanol in vivo regulated Th cell differentiation and Th cytokine profiling, IgG2a and IgE production was measured in the serum of NOD mice. As expected, high levels of IgE and some decline in the levels of IgG2a were observed in the serum (Chang et al. 2004).

Since cytopiloyne (**15**) had the most potent effect on Th cell differentiation among the aforesaid polyynes (Chang et al. 2007), another study used cytopiloyne

to explore the action and molecular mechanism of cytopiloyne on T1D in NOD mice (Chang et al. 2007). NOD mice received intraperitoneal or intramuscular injection of cytopiloyne at 25 μg/kg BW, 3 times per week. Twelve-week-old NOD mice started to develop T1D, and 70% of NOD mice aged 23 weeks and over developed T1D. Remarkably, 12- to 30-week-old NOD mice treated with cytopiloyne showed normal levels of blood glucose (<200 mg/dL) and insulin (1–2 ng/mL). Consistent with T1D incidence, cytopiloyne delayed and reduced the invasion of $CD4^+$ T cells into the pancreatic islets (Chang et al. 2007). Albeit less effectively than cytopiloyne (**15**), 3-ß-D-glucopyranosyl-1-hydroxy-6(*E*)-tetradecene-8,10,12-triyne (**19**), 2-ß-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7,9,11-triyne (**17**) also decreased T1D development in NOD mice.

The underlying mechanism by which cytopiloyne and its derivatives inhibited T1D included inactivation of T cells, polarization of Th cell differentiation, and Th cell depletion, leading to islet protection (Chang et al. 2007) as is illustrated in Fig. 6.

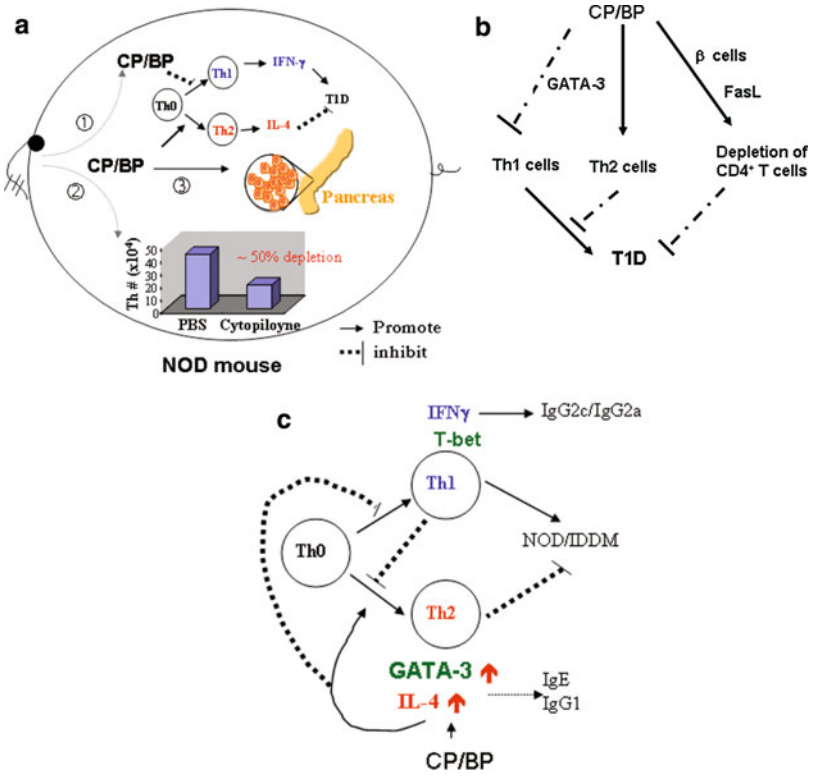


Fig. 6 The underlying mechanism of the crude extract of *B. pilosa* (BP) and its active polyyne, cytopiloyne (CP, **15**), in T1D. BP and/or CP can suppress T1D development via regulation of T cells (① and ②) and β cells (③) in NOD mice (**a**). Their regulation of T cells involves Th cell activation and differentiation (①) and partial depletion of Th0 cells (②) as depicted (**b**). CP and/or BP augment the expression of GATA-3 gene and, in turn, promote the expression of IL-4 and Th2 cell differentiation. In contrast, BP and/or cytopiloyne (CP) do not affect the expression of T-bet (**c**)

First, [^{3}H] thymidine incorporation assay showed that cytopiloyne inhibited ConA/IL-2- and CD3 antibody-mediated T cell proliferation, implying that cytopiloyne could inhibit T cell activation. Second, in vitro study showed that cytopiloyne (**15**) inhibited the differentiation of naïve Th (Th0) cells (i.e., $CD4^+$ T cells) into Th1 cells and promoted differentiation of Th0 cells into Th2 cells (Chiang et al. 2007). The in vitro data are consistent with the in vivo results indicating that cytopiloyne reduced Th1 differentiation and increased Th2 differentiation as shown by intracellular cytokine staining and FACS analysis (Chang et al. 2007). Cytopiloyne also enhanced the expression of GATA-3, a master gene for Th2 cell differentiation, but not the expression of T-bet, a master gene for Th1 cell differentiation, further supporting its role in skewing Th differentiation (Chang et al. 2007). In line with the skewing of Th differentiation, the level of serum IFN-γ and IgG2c decreased while that of serum IL-4 and serum IgE increased compared to the negative controls (PBS-treated mice). Third, cytopiloyne partially depleted $CD4^+$ rather than $CD8^+$ T cells in NOD mice (Chang et al. 2007). Co-culture assays showed that the depletion of $CD4^+$ T cells was mediated through the induction of Fas ligand expression on pancreatic islet cells by cytopiloyne, leading to apoptosis of infiltrating $CD4^+$ T cells in the pancreas via the Fas and Fas ligand pathway. However, cytopiloyne did not induce the expression of TNF-α in pancreatic islet cells and, thus, had no effect on $CD8^+$ T cells (Chang et al. 2007). Collectively speaking, the mechanism of action of cytopiloyne and, probably, its polyyne derivatives, in T1D, includes inhibition of T-cell proliferation, skewing of Th cell differentiation and partial depletion of Th cells and protection of β pancreatic islets (Chang et al. 2007; Chiang et al. 2007).

43.5 Benefits (human studies)

43.5.1 Cancer

C. albida, *G.korsiensis*, *V. scorpioides*, *N. incisium*, *M. maingayi*, *C. latifolia*, *A. cordata*, *P. ginseng*, *B. pilosa*, and *P. stipueantus* have been used as traditional medicines or ethnomedicines to treat cancer and vasculature diseases in humans. Currently, their polyynes are not clinically available for humans. In contrast, falcarindiol, falcarinol, falcarindiol-3-acetate, and falcarinone are rich in plants that are used as food such as carrot, celery, etc. and are considered to be nutraceuticals for patients with cancer. However, falcarindiol, falcarinol, and their derivatives need to be evaluated in clinical trials.

43.5.2 Metabolic Syndrome

Over 1200 plant species have been claimed to treat diabetes (Habeck 2003; Marles and Farnsworth 1995). *B. pilosa* and *P. ginseng* have been used to treat different categories of illness in humans. For example, *B. pilosa* formulation (probetacell) was shown to reduce the level of FBG and HbA1c in patients with diabetes but increased fasting serum insulin in healthy subjects (Lai et al. 2015). Moreover, a combination

of *B. pilosa* formulation with antidiabetic drugs had better glycemic control in diabetics (Lai et al. 2015). The homeostatic model assessment (HOMA) data suggested that the antidiabetic activity of this formulation was via improvement of β-cell function (Lai et al. 2015). Furthermore, the toxicological studies showed that oral administration with the *B. pilosa* formulation at a daily dose of 400 mg per 70 kg body weight, three times a day for 3 months, had no obvious side effects in healthy subjects (Lai et al. 2015). The overall data concluded that *B. pilosa* has potential as an antidiabetic remedy. However, the efficacy of the polyynes from *B. pilosa* and *P. ginseng* in treating metabolic syndrome in human subjects is not clear.

43.5.3 Infectious Diseases

C. Canadensis, *L. nudicaulis*, *H. annuus*, *V. scorpioides*, *M.aurea*, *B. pilosa*, *X. subacaulis*, *P. evecta*, *P. suberoa*, and *Mkilua fragrans* have been used as herbal medicines for human infections. However, the potential of their polyynes to treat viral, bacterial, and fungal infections needs to be investigated.

43.5.4 Immune Diseases

A. dracunculus, *A. capillaris*, *A. macrocephala*, *B. parviflora*, *B. bipinnata, B. pilosa, C. tinctoria*, *E. atrorubens* and *E. pallida*, and *O. elatus* have a long history of human use. Only the *B. pilosa* formulation was clinically evaluated in humans (Lai et al. 2015). However, the potential of their polyynes to treat inflammatory disorders and immune diseases needs to be corroborated.

43.6 Application in Food (Including Correct Cooking of Foods Rich in Phytochemicals)

Dietary polyynes from edible plants might be more heat-labile than the other phytochemicals with different structures. Thus, cooking time needs to be considered.

43.7 Safety: Toxicity and Side Effects

Edible plants of the Asteraceae, Apiaceae, and other families regularly produce polyyne metabolites. Some plants and their polyynes have beneficial functions in humans. For example, carrot, celery, lettuce, parsley, parsnip, fennel, aubergine, artichoke, tomato, caraway, and *Bidens pilosa* are generally recognized as safe (GRAS). However, the others may have adverse effects. Thus, toxicological studies need to be tested for the plants and their polyynes without safety information. Polyynes are sometimes thought to have adverse effects in humans because long polyynes are inherently unstable, albeit very reactive, due to their chemical properties. Two enediynes derived from bacterial compounds, neocarzinostatin, and

calicheamicin/esperamicin, were approved as prescription drugs for cancer (Classes of enediynes, Wikipedia 2016) (Maeda 2001; Maiese et al. 1989; Golik 1987). However, despite good anticancer efficacy, both have fatal toxicity, which limit their clinical use.

In the case of *B. pilosa*, the Food and Agriculture Organization and Taiwanese Ministry of Health and Welfare allow its use as an ingredient in food for human consumption. Despite lack of systemic toxicological studies of *B. pilosa* in humans, some information about acute and/or sub-chronic toxicities has been reported in rodents. Frida and colleagues reported that one single oral dose of the water extract of *B. pilosa* leaves at 10 g/kg BW showed no obvious mortality or changes in the appearance of rats (Frida et al. 2007). The same extract at 0.8 g/kg BW/day, once a day, showed no obvious sub-chronic toxicity in rats over 28 days, as shown by survival rate, body weight, and gross examination of organs (Frida et al. 2007). They also evaluated the acute toxicity of hydroethanol extracts of *B. pilosa* in mice (Frida et al. 2007). Five- to six-week-old mice, weighing between 28 and 35 g, received a peritoneal injection of both extracts at different doses. The LD_{50}, the dose that causes 50% lethality, of the hydroethanol extracts in mice was 12.3 g/kg BW and 6.2 g/kg BW, respectively (Frida et al. 2007). Ezeonwumelu et al. showed that oral delivery of the water extract of the *B. pilosa* whole plant at 1 g/kg BW/day, once a day, seemed nontoxic in rats over 28 days in Wistar rats (Ezeonwumelu et al. 2011), which is in line with our observations in rat (Bartolome et al. 2013). Overall, these data suggest that consumption of *B. pilosa* aqueous extract at up to at 1 g/kg BW/day, once a day, is highly safe in rats. A complete toxicology and drug-drug interaction of *B. pilosa* with other drugs in humans are required prior to its further medical use.

43.8 Marketed Products

Juice and dried products of carrot, celery, lettuce, parsley, parsnip, and tomato are commercialized and consumed worldwide. Moreover, nutraceuticals and/or therapeutics of *P. ginseng* and *B. pilosa* have been already commercialized on the market in the world.

43.9 Patents

Dietary polyynes from *B. pilosa* have been patented for treatment of metabolic syndrome (Patent US2011269702 A1), cancer (Patent US20110280809A1), and protozoan infections (Patent US 9072312B2) in humans and animals.

43.10 Perspectives

Edible plants of the Asteraceae, Apiaceae, and other families regularly produce polyyne metabolites. They are used as foods, drinks, and medicines for human health. Polyynes may have beneficial effects in humans. However, some of them

may have adverse effects. Thus, toxicological studies are needed to test the plants and their polyynes for which there is no safety information to date. Precautions and contraindications in the nutraceutical and/or therapeutic use of the plants and their polyynes are needed.

43.11 Cross-References

- ▶ Antioxidants in Diets and Food
- ▶ Biflavonoids and Oligomeric Flavonoids from Food
- ▶ Dietary Coumarins
- ▶ Dietary Diterpenoids
- ▶ Dietary Monoterpenoids
- ▶ Dietary Triterpenoids
- ▶ Flavonoid C-Glycosides in Diets
- ▶ Lignans in Diets
- ▶ Sesquiterpenes in Cereals and Spices
- ▶ Sesquiterpenes in Fresh Food

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Abstract

Starch, a common constituent of higher plants, is the major form in which carbohydrates are stored. This chapter first introduces chemistry structure, synthesis, digestion, metabolism, and bioavailability of starch. Based on its digestion rate and extent, starch is classified into rapidly digestible starch, slowly digestible starch, and resistant starch. Resistant starch cannot be digested in the small intestine but can be fermented in the large intestine. This chapter introduces five kinds of resistant starch and commercially manufactured products and describes the fermentation process of resistant starch in detail, including the metabolism pathways, the bacteria involved, and end products. The fermentability of resistant starch depends on its physical and chemical structure. Particularly, short-chain fatty acids, mainly acetate, butyrate, and propionate, are produced during fermentation of resistant starch. These short-chain fatty acids have considerable bioactives. As a result, consumption of resistant starch has many benefits, including the prebiotic effect, decreasing protein fermentation, keeping colon healthy, the hypoglycemic effect, the anti-obesity effect, reducing inflammation and oxidative stress, improving mineral absorption, etc.

Keywords

Starch · Low digestion · Resistant starch · Fermentation · Gut bacteria · Colon health

44.1 Introduction

Starch, a common constituent of higher plants, is the major form in which carbohydrates are stored. It can be deposited in roots, tubers, fruits, seeds, etc. Humans and their ancestors always eat starchy foods derived from roots, tubers, fruits, or seeds (Miao et al. 2018). It is suggested that starch is of great importance for human evolution (Hardy et al. 2015). In addition, starch is widely used in our daily life. The history of starch is well documented by Schwartz and Whistler (2009). The use of starch products may date back to the pre-dynastic period when Egyptians cemented strips of papyrus together using starch adhesive made from wheat. Later, sheets were first coated with a high-fluidity starch to prevent ink penetration and then covered with powdered starch to improve their weight and thickness in China. Nowadays, Chinese people still use the starch paste made form rice gruel to stick the documents such as couplets on the wall, which is a tradition since a very long time ago. In the Middle Ages, several starches and starch-based products, such as wheat starch, potato starch, maize starch, dextrin, and starch syrups, appeared. The starch industry enormously expanded in the nineteenth century, largely due to demands of the textile, paper, and color printing industries and the emerging of dextrin. By the 1930s, numerous starch products were developed by carbohydrate chemists, which greatly expanded the application of starch.

Table 1 Roles starches play in various food systems (Mason 2009)

Functions	Foods
Adhesion	Battered and breaded foods
Binding	Formed meat, snack seasonings
Clouding	Beverages
Crisping	Fried and baked foods, snacks
Dusting	Chewing gum, bakery products
Emulsion stabilization	Beverages, creamers
Encapsulation	Flavors, beverage clouds
Expansion	Snacks, cereals
Fat replacement	Ice cream, salad dressings, spreads
Foam stabilization	Marshmallows
Gelling	Gum drops, jelly gum centers
Glazing	Bakery, snacks
Moisture retention	Cakes, meats
Thickening	Gravies, pie fillings, soups

Due to sustainability, biodegradability, biocompatibility, edibility, and low cost, starch is one of the most widely used raw materials in food, textile, and pharmaceutical industries. Unfortunately, native starches usually have some defects, which restrict their applications. Therefore, starch is usually modified physically, chemically, and/or enzymatically to enhance their positive attributes and/or to minimize their defects (Miao et al. 2018). For instance, pregelatinization achieves rapid dissolving of starch or starchy foods. Dextrinization increases water solubility of starch. Carboxymethyl starch has higher freeze-thaw stability, and cross-linking starches have greater resistance to stress. Therefore, modification extends application of starch. Generally, starch and starch derivatives are widely used in food products and play important roles, such as gelling agents, thickeners, emulsifying agents, and encapsulating agents (Table 1) (Mason 2009). Starch is also used in papermaking as wet-end additives for dry strength, surface sizes, and coating binders and as adhesives for warp sizing of textiles and glass fiber sizing (Chiu and Solarek 2009). Modified starches are the common ingredient in tablets. In recent years, some starch is used to make the plastic product due to its biodegradability. Therefore, the starch production is very large and increasing each year. The global annual production of pure native starch reached 73 MT in 2011 and was expected to reach 133.5 MT in 2018 (Blennow 2018).

44.2 Chemistry

44.2.1 Chemical Structure of Starch

Starch exists in the form of semicrystalline granules that consist of amylose and amylopectin with very small quantities of proteins, minerals, lipids, and ash. Amylose accounts for 20%–30% by weight in most native starches (Hu et al. 2018; Miao

et al. 2018); expect that waxy starches do not contain amylose. In recent years, genetic strategies have been used to enhance the amylose content in some starches. For instance, high-amylose maize starches have been developed, and there are commercial products, such as amylomaize V, VI, and VII, which correspond to the amylose content of approximately 50%, 60%, and 70%, respectively (Vineyard et al. 1958; Jiang et al. 2010). Amylopectin, the major component of most starches, is a complex branched polysaccharide. The degree of polymerization (DP) of amylopectin ranges 3×10^5–3×10^6 (Zobel 1988). The α-D-glucopyranosyl residues of chains are linked mainly by α-1,4-linkages, and these chains are linked together by α-1,6 bonds at the branch points to form branches (Buléo et al. 1998). Approximately 5%–6% of the glucosyl units in amylopectin are joined via (1-6) bonds, which introduce chain branches. The amylopectin chains are classified into A-, B-, and C-chains as defined by Peat et al. (1952). Each amylopectin molecule has one single C-chain (Pérez and Bertoft 2010). The C-chain contains the terminal reducing end oriented towards the center or hilum of the granule and carries other chains as branches. The B-chains are attached to the C-chain by α-1,6-linkages. The A-chains are the outer chains without any branches which are glycosidically linked at their potential reducing group through C6 of a glucose residue to B-chains. The B-chains carry A- or B-chains as branches. A- and B-chains form clusters and B-chains can carry multiple clusters. These chains are always different in DP, which leads to a broad distribution of the chain length. A-chains typically consist of 6–12 glucosyl units, while B-chains usually contain more glucosyl units. It is well-known that the chain length of amylopectin significantly affects the physicochemical properties. Therefore, chain length distribution is one of the key characteristics for amylopectin. Amylopectin structure usually varies between species and even differs in organelles within the same species (Jaiswal and Chibbar 2017). Potato starches carry more long chains than other starches (Semeijn and Buwalda 2018). The chain length distribution is usually analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection and fluorophore-assisted capillary electrophoresis.

Some native starches, particularly potato starch, possess phosphorylation, and the phosphate groups are mostly monoesterified at the C-3 and C-6 positions of the anhydrous glucose residues of amylopectin in the amorphous parts of the starch granules (Hizukuri et al. 1970). The C-6 phosphate esters are in majority and account for approximately 70% of the total phosphorylation (Hizukuri et al. 1970; Tabata and Hizukuri 1971). These phosphate groups generate charge on the starch molecules, providing the starch with low temperature of gelatinization. Moreover, the starch paste is relatively clear with high viscosity.

Amylose was defined as a linear molecule whose α-D-glucopyranosyl units were linked by α-1,4-linkages, but today it is recognized that some amylose molecules are slightly branched by α-1,6-linkages like amylopectin. The DP of amylose ranges 1500–6000, which is much smaller than amylopectin (Zobel 1988). It is generally accepted that the crystalline part of starch granules is composed of double helices formed by side chains of amylopectin, while the branching point of amylopectin and amylose is located in the amorphous region. Both amylopectin and amylose can

interact with I_2 to form complex. The color and intensity of the starch-I_2 complex depend on the chain length of amylopectin or length of amylose (Baldwin et al. 1944). The amylose-I_2 complex is blue, and its maximum absorbance wavelength (λ_{max}) is approximately 620 nm. The color of the amylopectin-I_2 complex shifts to red-purple, and the λ_{max} shifts to lower wavelengths at 530–575 nm, since the chain length of amylopectin is much smaller than the length of amylose. The blue value (BV) which indicates the complex ability of starch and I_2 is defined as the absorbance at 680 nm of 1 mg starch in 100 mL solution containing 2 mg I_2 and 20 mg KI. Absolutely, amylose has higher BV at 1.01–1.63, whereas BV of amylopectin is low, ranking 0.08–0.38 (Bertoft 2018). Besides, amylopectin and amylose display other different properties, such as viscosity and crystallization behavior. Due to the difference of amylopectin and amylose, the amylose content is an important parameter of native starch.

44.2.2 Starch Synthesis

Amylopectin biosynthesis is executed by a coordinated series of enzymes, including ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SS, including SSI, SSII, SSIII, and SSIV), and starch branching enzyme (BE, including BEI and BEII), whereas amylose is synthesized by AGPase and granule-bound starch synthase (GBSSI) (James et al. 2003; Jaiswal and Chibbar 2017). The general scheme of starch synthesis is summarized in Fig. 1 (Buléo et al. 1998). Glucose is first phosphorylated into α-glucose-6-P (P represents the phosphate group) via action of hexokinase, and then α-glucose-6-P is converted into α-glucose-1-P via action of phosphoglucomutase. Alternatively, α-glucose-1-P results from phosphorolytic degradation of starch, which is catalyzed by starch phosphorylase. The α-glucose-1-P should be activated into adenosine diphosphate-glucose (ADP-Glu), the glucosyl

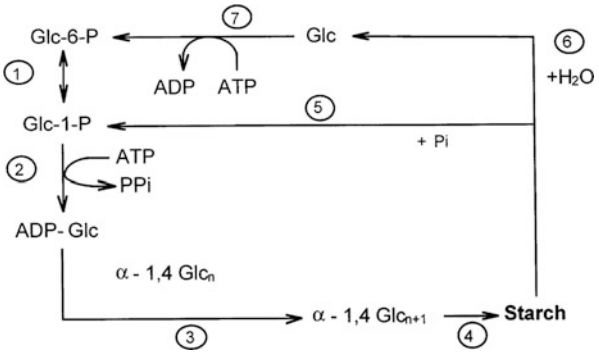

Fig. 1 General scheme for starch biosynthesis: (1) phosphoglucomutase; (2) ADP-glucose pyrophosphorylase; (3) granule-bound and soluble starch synthases; (4) branching enzymes; (5) starch phosphorylase; (6) amylases, branching enzymes, maltases; (7) hexokinase (Buléo et al. 1998). ADP represents adenosine diphosphate and ATP represents adenosine triphosphate

donor. This reaction is catalyzed by AGPase. Then, the glucosyl unit of ADP-glucose is transferred to the nonreducing end of a glucan chain by formation of α-1,4 glycosidic bond for elongation of linear glucan chain, which is catalyzed by SS or GBSS. Studies on roles of SS isoforms in chain length distribution of amylopectin indicate that SSI is primarily responsible for the synthesis of shortest chains (DP $\leq$ 10) (Keeling and Myers 2010). The longer chains of amylopectin are mostly synthesized by SSII and SSIII (Commuri and Keeling 2001). Accordingly, differences in contribution of starch synthases have been observed with respect to species and even different tissues in the same species, which cause variations in fine structure of amylopectin (Smith et al. 1997). The branches of the amylopectin and amylose molecules are produced by SBE, which cleaves internal α-1,4 glycosidic linkage and attaches the released chain through an α-1,6 glycosidic bond to a new site on the glucan molecule. BEI and BEII differ in terms of the lengths of chains transferred in vitro (James et al. 2003). Specifically, BEII transfers shorter chains than BEI. In another study, it was found that BEs might act sequentially during starch synthesis; BEII acted first and produced precursors which further acted as substrate for BEI (Seo et al. 2002). Mutations in many species suggest that starch synthesis also involves debranching enzymes (DBEs) (James et al. 2003). Two DBE families exist in plants, isoamylase type and pullulanase type. They hydrolyze α-1,6 bonds but differ in substrate specificity. Final packaging of starch granules requires trimming of extra branches, and DBEs play this role (Ahuja et al. 2013). Two mechanisms for DBE mode of action have been proposed. According to the preamylopectin-trimming model, the outer branches of preamylopectin molecules are trimmed by DBE to facilitate elongation of chains by SS. This results in amylopectin with an ordered branch structure and allows the molecules to package in starch granules. In addition, the glucan chains released by action of DBE on amylopectin can be used to form the amylose fraction by elongation action of GBSSI. According to the soluble glucan recycling model, DBE participates in degradation of short-chain glucans produced either by SS or SBE action to prevent accumulation of highly branched soluble polymers. This model is supported by the fact that phytoglycogen instead of amylopectin from soluble glucans is formed in endosperms deficient in DBE activity by lesions in DBE genes.

44.2.3 Modification of Starch

Food-grade enzymes, such as α-amylase, β-amylase, amyloglucosidase, and pullulanase, are used to produce maltodextrin, modified starches, or syrups (Jiang et al. 2014; Li et al. 2014; Qi et al. 2017). α-Amylase randomly breaks down the inner α-1,4 glycosidic bonds of starch (Miao et al. 2014d). β-Amylase acts from the nonreducing end of starch and hydrolyzes the second α-1,4 glycosidic bond, cleaving off two glucose units at a time and producing maltose (Miao et al. 2014c). But it cannot pass α-1,6 branch linkage. Therefore, only approximately 40%–60% of amylopectin is converted to maltose, and the remaining part is the β-limit dextrin (Tester and Qi 2011). Amyloglucosidase catalyzes the hydrolysis of both α-1,4 and

α-1,6 bonds, but the rate of hydrolyzing α-1,6 bond is much slower (Miao et al. 2014b). Pullulanase is often used to debranch starch due to its specificity on α-1,6 bond (Miao et al. 2009).

Every α-D-glucopyranosyl unit of starch molecules has three hydroxyl groups, which provides active sites for chemical modification (Lu et al. 2016). Generally, chemical modification of starch involves oxidation, etherification, esterification, or cross-linking of the available hydroxyl groups on the α-D-glucopyranosyl units of starch molecules; thus new groups are introduced to the starch polymer (Miao et al. 2011). Examples of esterified starches include hydroxypropyl starch, hydroxyethyl starch, or carboxymethyl starch, whose hydroxyl groups are partially substituted by hydroxypropyl, hydroxyethyl, or carboxymethyl group through the formation of an ether link (R-O-R), respectively (Masina et al. 2017). Starch octenyl succinate and starch acetate are generally obtained by the esterification of native starch with octenyl succinic anhydride (OSA) and acetic anhydride in the presence of an alkaline catalyst, respectively (Miao et al. 2014a). Cross-linking modification is intended to randomly produce intra- and intermolecular bonds between hydroxyl groups of starch. The commonly used agents to cross-link food-grade starches include sodium trimetaphosphate, sodium tripolyphosphate, monosodium phosphate, phosphoryl chloride, epichlorohydrin, vinyl chloride, and a mixture of adipic acid and acetic anhydride (Singh et al. 2007).

44.3 Metabolism and Bioavailability

44.3.1 Metabolism of Digestible Starch

Starch must be digested into glucose to be absorbed in the small intestine of human beings. Starch is firstly hydrolyzed by salivary α-amylase in oral cavity. Chewing comminutes the food and provides good interaction between salivary α-amylase and starch, which may protect the enzyme inside the bolus and continue to digest starch to some extent in the low-pH environment of the stomach. After the journey through the stomach, starch arrives at the small intestine and is hydrolyzed by pancreatic α-amylase. At this point, starch is hydrolyzed into glucose, maltose, maltotriose, α-limit dextrin, isomaltose, etc. Then these non-glucose molecules are further hydrolyzed into glucose by α-glycosidases at the brush border of the small intestine, including maltase-glucoamylase and sucrase-isomaltase. Maltose, maltotriose, and maltotetraose are hydrolyzed into glucose by maltase-glucoamylase via successive action from their nonreducing end. The α-1,6-linkages of hydrolyzed starch are hydrolyzed by sucrase-isomaltase. Finally, glucose can be actively transported across the enterocyte of the small intestine via the sodium glucose cotransporter 1 (SGLT-1) and enters the blood, thus increasing the blood glucose concentration.

The increasing of the blood glucose concentration leads to secretion of insulin, and the insulin facilitates tissue uptake of glucose to decrease the blood glucose concentration. Some glucose molecules are oxidized immediately to provide energy,

and some are used to synthesize glycogen in liver and muscle tissues. However, the capacity to synthesize glycogen for storing glucose of human body is limited. The maximum storage capacity for storing glucose by glycogen is approximately 700 g (Li 2018). This capacity in muscle is in majority. However, this capacity in the liver is limited, and only a maximum of approximately 150 g glucose can be stored in the liver of a normal 70 kg person. If the glucose intake exceeds both the oxidative and glycogen storage capacities, glucose will be converted into fat. The capacity to convert glucose into fat is much larger, which can explain why eating too much induces obesity. Liver glycogen can be degraded into glucose, and glucose is released to general circulation. Glycogenolysis which means degradation of glycogen into glucose functions according to the body's needs, which plays an important role in maintaining blood glucose levels constant during the intervals between meals (Blanco and Blanco 2017). However, muscle glycogen does not release glucose into the general circulation. Actually, muscle glycogen serves as an energy reserve for this tissue and is intensely utilized when muscle performs work, such as high-intensity exercise. In this case, breakdown of glycogen produces pyruvate and lactate in muscle.

The catabolism of glucose mainly takes place through glycolysis (also known as Embden-Meyerhof pathway), which is fully completed in the cell cytoplasm (Blanco and Blanco 2017). This pathway includes ten reactions (Fig. 2). Phosphorylation is the initial step for the metabolic utilization of glucose (Reaction (1)). The first metabolic transformation is the esterification with phosphate to form glucose-6-P. This reaction is catalyzed by hexokinase, an enzyme present in all cells, or glucokinase. The formation of glucose-6-P is important for converting glucose into a more reactive compound, which is suitable for further transformations. In addition, because glucose-6-P cannot pass through cell membranes, glucose is trapped into the cell via glucose phosphorylation. Moreover, rapid conversion of glucose to glucose-6-P maintains the intracellular glucose concentration at a low level, which facilitates the continual entry of glucose into the cell. In Reaction (2), the aldo sugar, glucose-6-P, is isomerized to the keto sugar, fructose-6-P. This reaction is catalyzed by phosphoglucoisomerase. Then, fructose-6-P is further phosphorylated at the other end by phosphofructokinase to generate fructose-1,6-bisphosphate (fructose-1,6-P_2) (Reaction (3)). This reaction requires the transfer of a phosphoryl group from ATP and is catalyzed by phosphofructokinase. In Reaction (4), fructose-1,6-P_2 is cleaved into two triosephosphate molecules: glyceraldehyde-3-P and dihydroxyacetone-P. Then, dihydroxyacetone-P is transformed into glyceraldehyde-3-P (Reaction (5)). These steps are usually considered as the first or early phase of glycolysis. In Reaction (6), glyceraldehyde-3-P is oxidized and phosphorylated into 1,3-bisphosphoglycerate. This reaction is catalyzed by glyceraldehyde- 3-phosphate dehydrogenase, an oxidoreductase that uses nicotinamide adenine dinucleotide (NAD^+) as coenzyme. Therefore, the acceptor of the reducing equivalents is NAD^+, and it becomes reduced nicotinamide adenine dinucleotide (NADH), while the second hydrogen is released simply as a proton in solution. Substrate-level phosphorylation occurs in Reaction (7). High-energy phosphate is transferred from 1,3-bisphosphoglycerate to ADP, which is catalyzed by phosphoglycerate kinase.

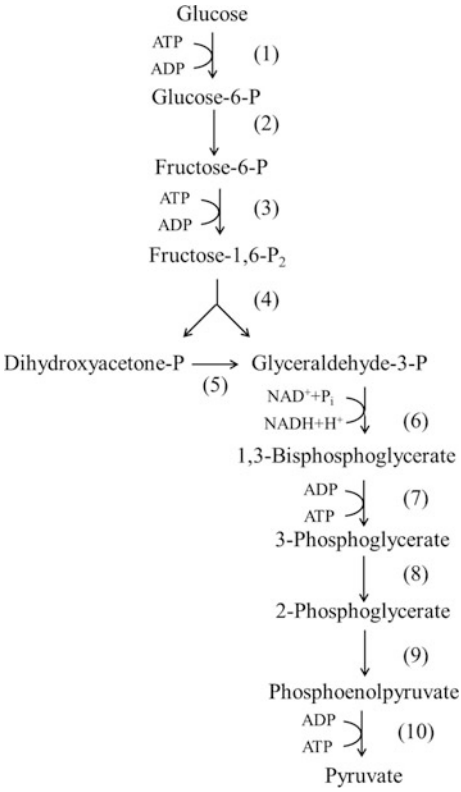

Fig. 2 Reactions of glycolysis

As a result, 3-phosphoglycerate and ATP are produced. Subsequently, 3-phosphoglycerate is converted into 2-phosphoglycerate via an intramolecular phosphoryl transfer (Reaction (8)), which is catalyzed by phosphoglycerate mutase. 2-Phosphoglycerate is dehydrated and intramolecularly redistributed, generating an energy-rich compound, phosphoenolpyruvate (Reaction (9)). The second substrate-level phosphorylation occurs in Reaction (10). The energy-rich phosphoenolpyruvate transfers a phosphate molecule to ADP, forming ATP and pyruvate. This reaction is catalyzed by pyruvate kinase.

The early steps of glycolysis actually consume two ATPs, but four ATPs are produced in the later reactions, resulting in a net production of two ATPs per glucose molecule. Therefore, glucose can quickly provide energy through glycolysis. In this catabolism pathway, two pyruvate molecules are obtained by cleaving a glucose molecule. The fate of pyruvate depends on the oxidative state of the tissue. For glycolysis to proceed, the NADH produced in Reaction (6) must be reoxidized back to NAD^+. Under aerobic conditions, the reducing equivalents from NADH are transferred to the mitochondrial electron transport chain and ultimately to molecular oxygen (Tornheim 2018). In this case, pyruvate produced from glycolysis is completely oxidized to CO_2 and H_2O, which is a complicated process. Pyruvate is first decarboxylated, which produces CO_2 and acetyl-coenzyme A (acetyl-CoA).

Acetyl-CoA moves into the citric acid cycle (also known as tricarboxylic acid (TCA) cycle or Krebs cycle), being completely oxidized to CO_2. The resultant reducing equivalents are transferred to the mitochondrial electron transport chain and ultimately to molecular oxygen, producing H_2O and more ATPs. However, when there is insufficient oxygen or insufficient activity of the electron transport chain, pyruvate must be used to oxidize NADH and regenerate NAD^+ in the lactate dehydrogenase reaction. In this case, pyruvate is reduced to lactate. This is the reason why strong muscular exercise produces lactate. Lactate formation is very important for muscle because it can rapidly use the ATP generated through glycolysis to contract. Increased levels of lactate can be detected in blood and urine after intense exercise, which directly indicates the level of glycolytic activity of muscle. Alternatively, if there is excess use of glucose beyond what would be necessary for energy production, acetyl-CoA can also be used for synthesis of fatty acids, the main constituent of fat (Tornheim 2018). Absolutely, aerobic catabolism of glucose is much more efficient but much slower in energy production than anaerobic catabolism. Particularly, glycolysis is the only pathway for energy production in mature red blood cells due to lack of mitochondria. In addition, the intermediates from glycolysis and citric acid cycle play important roles in supplying the carbon backbone for synthesis of many cell constituents. For instance, 2,3-bisphosphoglycerate which is generated from 1,3-bisphosphoglycerate is an important modulator of hemoglobin. Glycerol-3-P which is formed from dihydroxyacetone participates in the synthesis of triacylglycerols and phospholipids.

In most tissues, 80% or more of glucose catabolism initially enters glycolysis. The rest follows another pathway called the pentose phosphate pathway or the hexose monophosphate pathway. It can be divided into two phases (Fig. 3). The first phase is the oxidative phase. Here glucose-6-P undergoes two oxidations and decarboxylation and is transformed into ribulose-5-P. First, glucose-6-P is dehydrogenated and produces gluconolactone-6-P. This reaction is catalyzed by glucose-6-P dehydrogenase, which depends on nicotinamide adenine dinucleotide phosphate ($NADP^+$) as the hydrogen acceptor. Therefore, reduced nicotinamide adenine dinucleotide phosphate (NADPH) is produced. Then, gluconolactone-6-P is converted into gluconate-6-P. In Step 3, gluconate-6-P is oxidized and transferred into ribulose-5-P and CO_2, which is catalyzed by another $NADP^+$-dependent enzyme, gluconate-6-P dehydrogenase. Thus, NADPH is produced again. The second phase is the nonoxidative phase, which comprises a series of reversible reactions. Firstly, ribulose-5-phosphate produces two isomers: ribose-5-P and xylulose-5-P. They are then transferred into glyceraldehyde-P and sedoheptulose-7-P via action of transketolase, which in turn generate fructose-6-P and erythrose-4-P via action of transaldolase. Erythrose-4-P and xylulose-5-P are redistributed to form glyceraldehyde-3-P and fructose-6-P via action of transketolase. Obviously, glyceraldehyde-3-P and fructose-6-P are intermediates of glycolysis and can enter the glycolysis pathway. In summary, this pathway produces two important substances: pentose phosphate and NADPH (Tornheim 2018). Pentose phosphate is a precursor for synthesis of nucleotides and nucleic acids. On the other hand, NADPH is indispensable to anabolism as a reducing agent, which is used in various

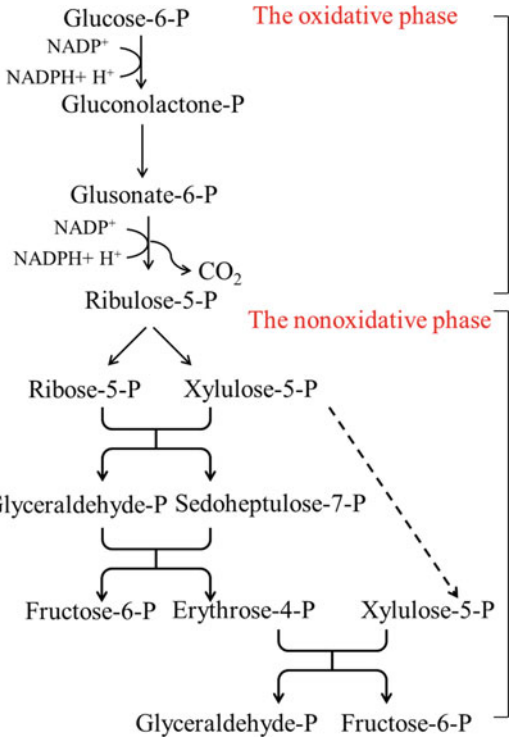

Fig. 3 The pentose phosphate pathway

processes, including fatty acid synthesis, regenerating reduced glutathione, cholesterol and bile acid synthesis, steroid hormone synthesis, and cytochrome P450-dependent biotransformation. Therefore, the pathway is very important for anabolism and is highly active in the tissues where these processes occur, such as liver.

44.3.2 Bioavailability of Starch According to Its Digestion Rate

Digestion of starch is affected by various factors that affect enzyme activity and the susceptibility of the starch substrate to the digestive enzyme (Miao et al. 2013, 2015b). For humans, enzyme activity is mainly affected by the starch structure and enzyme inhibitors either present in the food or generated during digestion of food. As stated above, the main hydrolysis of starch is performed by the α-amylases. Starch digestion by α-amylases requires a series of steps. The enzymes first have to diffuse to the starch matrix, then bind to starch, and finally cleave the α-1,4 glycosidic linkages. Physical entrapment of starch in the food matrix, starch granular structure, and crystallinity may affect the binding of enzymes to the starch substrate (Miao et al. 2015a). For starch granules, one of the limiting factors for the hydrolysis is the penetration of the enzyme into the granules by successive formation of pits and larger pores. On the other hand, the starch has to be properly oriented inside the

active side of the enzyme for the catalytic action of the enzyme to occur (Sun et al. 2019). Only the part of the starch that can fit into the active site cavity of the α-amylase can be hydrolyzed. Structures such as double helices are too big and rigid to fit into the active site cavity, which is another reason for why it is difficult for amylases to hydrolyze starch granules. Thus, the amorphous starch is less resistant to digestive enzymes than the crystalline starch. Similar phenomenon occurs to the starch-lipid complex with a left-handed helix, which is more resistant to enzymes. Molecular structure of starch also influences its digestion. As mentioned above, starch is comprised of amylopectin and amylose. Amylose is a linear polysaccharide whose glucose monomers are linked by α-1,4 glycosidic bonds, whereas the highly branched amylopectin contains α-1,6-linked glucose monomers that create branch points in addition to the linear α-1,4-linkages. The linear regions of the starch molecules are easily digested by human α-amylases. The digestion products of α-amylase, small sugar and oligosaccharide, are then digested further by the intestinal brush border enzymes. Therefore, humans hydrolyze α-1,6 glycosidic bonds more slowly than α-1,4 bonds. A linear portion of the starch chain has to fit in active site of α-amylase to be hydrolyzed. This linear portion must be long enough to favorably bind with the enzyme. Glucose units close to the branch points have less favorable binding with the active site of the enzyme, thus decreasing the hydrolysis efficiency. Thus, no matter what type of linkage that creates the branching of the starch chain, branch points create steric hindrance for α-amylase digestion. Chemical modification of starch usually introduces a bulky side group to the starch chains, which can be viewed as a modification that creates branches on the starch chains and brings steric hindrance to human digestive enzymes. Therefore, chemical modification including etherification, esterification, and cross-linking decreases the digestibility of starch. Particularly, cross-linking links two starch chains, in effect producing a branch on both starch chains.

Therefore, based on its rate and extent of digestion, starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al. 1992). RDS corresponds to the starch fraction digested within 20 min of incubation; SDS is the starch fraction digested within 20–120 min; and the remaining fraction is RS, which cannot be digested further in the small intestine (Miao et al. 2015a). Accordingly, RDS leads to a postprandial fluctuation in blood glucose with the blood glucose peak occurring 30–60 min after consumption (Hendrich 2018). For individuals with normal glucose metabolism, the blood glucose concentration is declined to the fasting state within ~2 h. Ingestion of SDS blunts and slows this pattern of blood glucose response. Ingestion of RS further blunts blood glucose response (Miao et al. 2015a).

44.3.3 Gut Bacterial Metabolism of RS

Generally, RS has been classified into four subtypes named RS1–RS4 (Englyst et al. 1992). RS1 refers to physically inaccessible starch that is enclosed in food matrixes. Starch granules are always surrounded by the protein matrix and cell wall materials.

For instance, it is confirmed that the protein matrix hinder digestion of rice starch (Ye et al. 2018). The whole cereal grains are digested much more slowly than flours, which suggests that the cell wall materials hinder digestion of rice starch. When cooked as whole kernels or coarsely ground seeds, the thick cell wall of legume seeds and the protein matrix in cereal grains prevent water penetration into the starch in the matrix (Birt et al. 2013). Therefore, starch granules do not have adequate moisture to fully swell and gelatinize. Without adequate swelling and gelatinization, starch is not readily susceptible to enzymatic hydrolysis. The cell wall material and the protein matrix also prevent enzymes from reaching and hydrolyzing starch through acting as a physical barrier. Examples of RS1-containing foods in the previous reports include pasta made from durum wheat by extrusion and breads made from whole or coarsely ground kernels of grains (Jenkins et al. 1988; Granfeldt et al. 1991). Durum wheat has a higher protein content and harder texture. Consequently, the postprandial glycemic response after ingesting semolina pasta is substantially lower than white bread. Residual starch that is not digested in the small intestine passes into the colon as RS1. However, these starches may become more accessible and less resistant after milling and chewing. RS2 is composed of native starch granules whose crystallinity makes them resistant to enzymatic hydrolysis. Examples of RS2 include uncooked potato starch, green banana starch, gingko starch, and high-amylose maize starch (Birt et al. 2013). However, after cooking, most of RS2 becomes highly digestible due to starch gelatinization and loss of the crystallites. An exception is high-amylose maize starch. This starch displays a high gelatinization temperature, above the boiling point of water. Therefore, under the common cooking conditions, this type of starch retains its crystalline structure and remains resistant to enzymatic hydrolysis. Retrograded starches formed after cooking belong to RS3. Examples include the starch found in cooked and cooled potatoes, bread crusts, cornflakes, and retrograded high-amylose maize starch. After cooked starchy foods are stored, particularly in a refrigerator, amylose molecules and long-chain branches of amylopectin are prone to forming double helices and lose their water-binding capacity. This process is called retrogradation. The double helices of starch molecules are resistant to amylases. Therefore, those factors which affect retrogradation of starch, such as the amylose content, chain length, and processing conditions, would influence the amount and quality of RS3.The amylose content is positively correlated with the RS3 yield. Several RS3 ingredients are in the market, which are usually derived from cooked and recrystallized maize or tapioca starch. Because amylose molecules have a greater tendency to retrograde than amylopectin molecules, high-amylose starch is often used to prepare RS3. To promote crystallization of starch and formation of RS3, the starch is usually debranched to increase the amount of linear chains (Maningat and Seib 2013). In addition, annealing and heat-moisture treatment can enhance the RS3 content, since more perfect structures are formed, resulting in an increase in enzyme resistance of the starch. RS4 includes chemically modified starches. The introduced groups, such as phosphate groups or octenyl succinic groups, partially inhibit the enzymatic hydrolysis of the starch molecule due to steric hindrance, resulting in RS.

Recently, a new type of RS, RS5, is proposed, which comes from the amylose-lipid complex (Ashwar et al. 2016). When starch interacts with lipids, the hydrocarbon chain of the lipid interacts with the hydrophobic moiety of the amylose chain and fills the central cavity of the amylose. The complex forms amorphous (Form I) or highly crystalline structures (Form II), which both show V-type crystallinity in the X-ray diffraction analysis. This complex occurs in small amounts to native starch, and its production can be enhanced by addition of exogenous fatty acids and heat processing, such as steam jet cooking, wet heat processing, and extrusion cooking (Panyoo and Emmambux 2017). The enzyme resistance of amylose-lipid complex is attributed to the helical conformation of the starch-lipid complex, which prevents amylose molecules from dispersing and interfering with enzymes for hydrolysis (Jane and Robyt 1984). Because the amylose-lipid complex is spontaneously formed during cooling after being heated above its dissociation temperature, RS5 is thermally stable (Panyoo and Emmambux 2017). In addition, the crystalline structure of the complex enhances its enzyme resistance. For instance, the crystalline amylose-lipid complex (Form II) is more resistant to amylolytic enzyme hydrolysis than the amorphous complex counterpart (Form I). The resistance of the complex also depends on the lipid structure. The complex made from longer length fatty acids has greater enzyme resistance, while fatty acids with a greater degree of unsaturation make the amylose-lipid complex with lower enzyme resistance (Hasjim et al. 2013).

Different from RDS and SDS, RS cannot be digested into glucose by the small intestine. Actually, RS passes through the upper digestive part and arrive at the colon where RS is fermented by gut bacteria. The fermentation products of RS by gut bacteria mainly include short-chain fatty acids (SCFAs, mainly acetate, butyrate, and propionate) and gases (methane, hydrogen, and carbon dioxide) (Birt et al. 2013). In addition, few branched-chain fatty acids (isobutyrate and isovalerate), organic acids (lactate, succinate, and formate), and alcohols (methanol and ethanol) are produced. Fermentation of RS is a cooperative process in the lower gut, including (1) degradation of starch polymers into glucose performed by amylolytic gut bacteria; (2) glycolysis with SCFAs or other organic acids as end products which is performed by butyrogenic bacteria; and (3) methane production by *methanogenic Archaea* spp. from formate, hydrogen, and carbon dioxide, the products of bacterial metabolism of RS (Flint et al. 2008).

A range of enzymes is involved in breakdown of RS in the gut, including α-amylases that cleave α-1,4-linkages, type I pullulanases that specifically hydrolyze α-1,6 bonds, and amylopullulanases that cleave both α-1,4 and α-1,6 bonds (Ramsay et al. 2006). By far, the greatest number of starch-degrading enzymes in the gut, including α-amylases, pullulanases, and amylopullulanases, belong to family 13 glycoside hydrolases (MacGregor et al. 2001). It was found that the majority of amylolytic isolates were identified as bifidobacteria (58%) and bacteroides (18%), and fusobacteria and butyrivibrios accounted for about 10% of starch-hydrolyzing bacteria isolated when using fresh feces to ferment soluble starch (Macfarlane and Englyst 1986). In another in vitro fermentation experiment, it was found that *Bifidobacterium* spp., *Bacteroides* spp., *Fusobacterium* spp., and strains

of *Eubacterium*, *Clostridium*, *Streptococcus*, and *Propionibacterium* could hydrolyze the gelatinized amylopectin and high-amylose maize starch, whereas only *Bifidobacterium* spp. and *Clostridium butyricum* could efficiently utilize ungelatinized high-amylose maize starch granules (Wang et al. 1999). Therefore, *Bifidobacterium* spp. and *Clostridium butyricum* would be particularly important to gut bacterial fermentation of insoluble starch. In addition, Ze et al. 2012 found that *Eubacterium rectale* and *Bacteroides thetaiotaomicron* showed limited ability to utilize RS2 and RS3 compared with *Bifidobacterium adolescentis* and *Ruminococcus bromii*. However, only *R. bromii* was proved to be able to stimulate RS2 and RS3 utilization by the other three bacterial species in co-culture, even in a medium that does not permit growth of *R. bromii* itself. These results suggested that *R. bromii* was a keystone species for degradation of RS in the human colon. A recent study in humans also confirmed that the primary degradation of RS2 was largely governed by features linked to *Firmicutes*, including *R. bromii* as a main taxon (Vital et al. 2018).

Bacterial binding to starch is important for fermentation of starch in some bacteria. The enzyme system in *B. thetaiotaomicron* responsible for soluble starch utilization has been well established (Reeves et al. 1996, 1997). The enzyme system is organized by an outer membrane protein complex, including starch-utilization-structure (Sus) gene clusters that bind to and hydrolyze starch. In the complex, these outer membrane Sus proteins regulate the binding and transporting products of starch from partial hydrolysis into the periplasm where they are hydrolyzed and processed further. Specifically, the membrane protein complex contains maltose-inducible outer membrane proteins, SusC, SusD, SusE, SusF, and SusG (Fig. 4) (Flint et al. 2008; Reeves et al. 1996, 1997). SusC and SusD are physically associated and majorly contribute to starch binding. It is likely that SusE and SusF also contribute to binding but not to the same extent as SusD. SusG seems to contribute little to starch binding but is essential for growth on starch. Starch-hydrolyzing activity in *B. thetaiotaomicron* is greatly cell-associated, with much of it being periplasmic. In *E. rectal*e, a complex which contains two glycoside hydrolase13 family enzymes and three ATP-binding cassette transporter solute-binding proteins at the cell surface is responsible for hydrolyzing starch and capturing the released maltooligosaccharides (Cockburn et al. 2015). A multi-domain cell wall-anchored amylase, one of the two enzymes, is tethered to the peptidoglycan layer and may bind the bacterium to starch via its five N-terminal carbohydrate-binding modules and one unknown domain. It preferentially targets starch or maltooligosaccharides longer than maltotriose. The main product is maltotetraose, and significant amounts of maltopentaose are also produced. The other enzyme is a membrane-associated maltogenic amylase, which breaks down maltooligosaccharides with higher DP than maltotriose. The three solute-binding proteins display a range of glycan binding specificities.

Recently, it is found that there is unique organization of extracellular amylases, which is called amylosomes, in the RS-utilizing human colonic *R. bromii* (Ze et al. 2015). Dockerin-cohesin interactions occur among the enzymes in the amylosomes. *R. bromii* is a specialized amylolytic bacterium belonging to the *Ruminococcaceae*, a

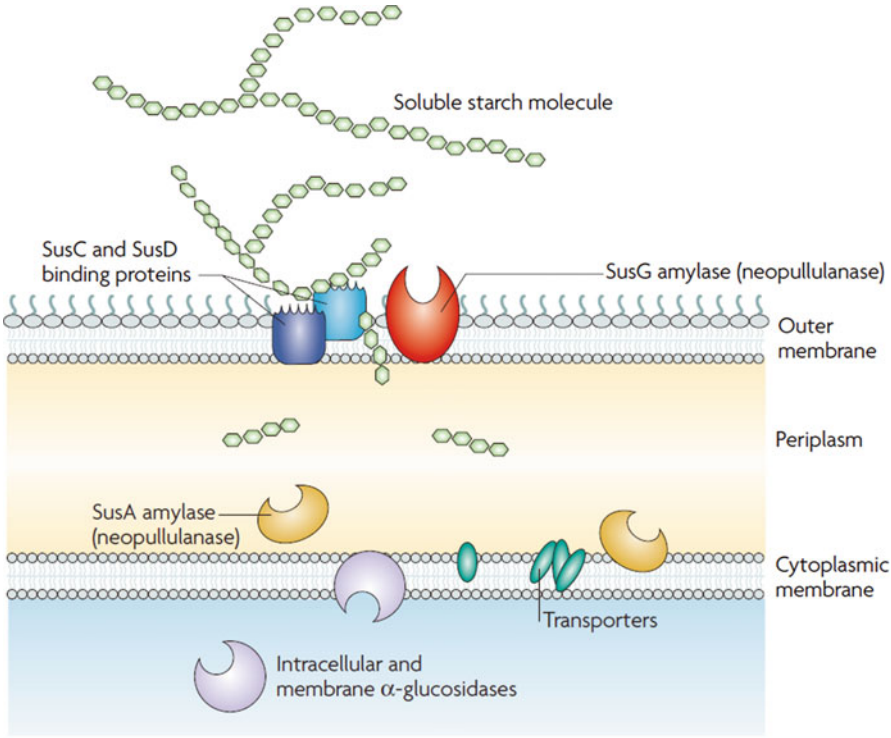

Fig. 4 The sequestration system for soluble starch in *B. thetaiotaomicron.* SusC and SusD are two proteins that have been shown to be essential in binding starch molecules to the cell surface. Limited hydrolysis by SusG is followed by more extensive hydrolysis in the periplasm and the uptake of oligosaccharides across the cytoplasmic membrane (Flint et al. 2008)

family of *Firmicutes* that is better known for the ability of certain rumen species to degrade cellulose. Compared with other amylolytic human intestinal bacteria such as *B. thetaiotaomicron*, *E. rectale*, and *B. adolescentis*, *R. bromii* shows high hydrolyzing activity against raw or boiled RS containing starch granules. It is observed that *R. bromii* cultures mainly have six extracellular GH13 amylases. They are four glycosidases, including Amy4, Amy1, Amy2, and Amy9, and two type I pullulanases, including Amy10 and Amy12. Amy4, Amy9, Amy10, and Amy12 carry dockerin, and Amy4 also carries a cohesin module. It is predicted that Amy4 and Amy9 bind a cohesin present in protein scaffolding. It is also inferred that further complexes are formed between the dockerin-carrying amylases Amy4, Amy9, Amy10, or Amy12 and two other cohesin-carrying proteins. In addition, Amy4 has the ability to autoaggregate, as its dockerin can recognize its own cohesin.

As stated above, glycolysis is the main pathway for glucose catabolism in human cells. Actually, glycolysis is a typical example of the unity of living organisms, since all living organisms have this route (Blanco and Blanco 2017). Many microorganisms metabolize glucose by this pathway through fermentation, but the end products

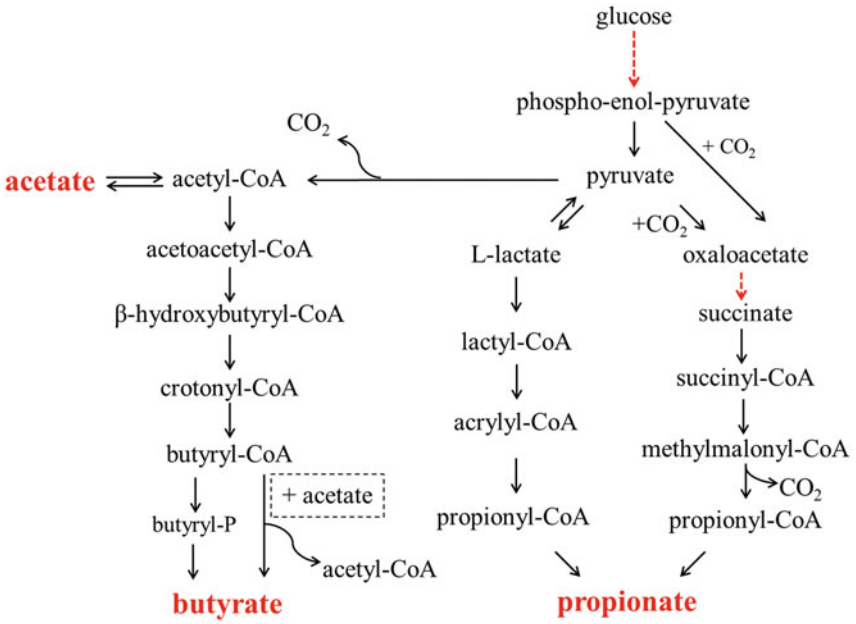

Fig. 5 Fermentation pathways leading to acetate, butyrate, and propionate formation from RS. Dotted arrow indicates several reactions

vary in different organisms. In the gut microorganisms, glycolysis is also the main pathway for glucose catabolism. After glycolysis, different pathways happen to pyruvate, resulting in different SCFAs. According to the previous reports (Pryde et al. 2002; Louis et al. 2007; Zhu et al. 2005), fermentation pathways leading to acetate, butyrate, and propionate are summarized in Fig. 5. Pyruvate is the major precursor of acetate and butyrate. Pyruvate is first decomposed into CO_2, one of the products for RS in gut, and acetyl-CoA. Acetyl-CoA is directly converted into acetate or participates in butyrate synthesis by butyrate kinase through a series of reactions. Acetate can act as a CoA acceptor and react with butyryl-CoA to synthesize butyrate, which is catalyzed by butyryl-CoA: acetate-CoA transferase. There are two routes to form propionate from glucose: the acrylate route from lactate is found in bacteria belonging to the clostridial cluster IX group, while *Bacteroides* species generally employ the succinate route to form propionate (Louis et al. 2007). Acetate accounts for approximately 60%–75% of the total SFCAs detected in feces and is formed by many of the bacteria, with around one-third coming from reductive acetogenesis (Miller and Wolin 1996). Butyrate-producing colonic bacteria generally belong to the clostridial clusters I, IV, XI, XIVa, XV, and XVI (Pryde et al. 2002). Two particularly abundant groups that together constitute 7%–24% of the total gut bacteria in healthy subjects are cluster IV bacteria related to *Faecalibacterium prausnitzii* and cluster XIVa bacteria related to *Eubacterium rectale* and *Roseburia* spp. The pathway employing butyrate kinase seems to widely

exist in different butyrate-producing *Clostridium* species and several clostridia (Zhu et al. 2005; Louis et al. 2007). The pathway employing butyryl-CoA:acetate-CoA transferase has been described in several bacteria, such as *Butyrivibrio fibrisolvens*, *Roseburia* sp., *F. prausnitzii*, and *Coprococcus* sp. (Diez-Gonzalez et al. 1997; Duncan et al. 2002).

Gas production is another fermentation outcome, and particularly the production of methane can be considered as the final end product consuming hydrogen and carbon dioxide (Li 2010). In ruminant animals, methanogens are ubiquitous. However, the distribution of methanogenic *Archaea* in human fecal bacterial populations is a good example of individual variability. Caucasians (48%) and Blacks (45%) had significantly more methane producers than Orientals (24%) and Indians (32%) by measuring breath hydrogen after lactulose intake (Pitt et al. 1980). On the other hand, it was reported that the abundance of methanogenic *Archaea* was negatively correlated to fecal butyrate concentration (Weaver et al. 1992; Belenguer et al. 2006). The production of methane mainly involves two parts as follows:

$$\text{Pyruvate} \rightarrow \text{CO}_2 + \text{H}_2 + \text{Formate}$$

$$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$$

The microbial communities of the large intestine are characterized by high cell densities and interspecies cross-feeding of fermentation products. Without exception, metabolic cross-feeding between bacteria plays an important role in metabolism of RS. For instance, the potential for metabolic cross-feeding between *B. adolescentis* and lactate-utilizing, butyrate-producing *Firmicute* bacteria related to *Eubacterium hallii* and *Anaerostipes caccae* was investigated in vitro (Belenguer et al. 2006). *E. hallii* L2-7 and *A. caccae* L1-92 failed to grow on starch in pure culture but produced butyrate when in co-culture with *B. adolescentis* L2-32, which confirmed cross-feeding of metabolites to the lactate utilizers. In summary, the utilization of RS is a complex and cooperative process in the gut. Identifying bacteria or bacterial functions in fermentation of RS is important for predicting health outcomes of ingesting RS (Li 2010).

Obviously, fermentation of RS is affected by many factors. It is generally accepted that the production of SCFAs, particularly butyrate, is considered to be favorable. Therefore, fermentability of RS is often reflected by the total SCFA production and the butyrate production. Obviously, the amount of RS entering the large bowel influenced the total and individual SCFA productions. As RS must be depolymerized by bacterial hydrolytic enzymes prior to fermentation, the rate of depolymerization affected the degree at which RS become available for bacteria. As stated above, only several bacteria can utilize starch granules. Thus, it can be inferred that fermentation of RS containing starch granules may be much slower than that of non-granular RS. In addition, granule dimension and surface area of RS containing starch granules may affect hydrolysis of RS. For example, it was found that hulless barley cultivar CDC Fibar (waxy starch) and CDC McGwire (normal starch) started to ferment sooner (lag time of 0.7 and 0.9 h, respectively) than

SH99250 (high-amylose starch; 1.7 h) (Jha et al. 2011). It was confirmed that total and individual SCFA productions also depended on its composition and physical structure of RS. For instance, Martin et al. (1998) found that luminal total SCFA in the caeco-colon of pigs 7 h after feeding potato starch (RS2), high-amylose maize starch (RS2), and retrograded high-amylose maize starch (RS3) were 33, 78, and 105 mmol, respectively, with potato starch providing the highest production of butyrate. They also demonstrated that in vivo fermentation of diets containing raw potato starch, high-amylose maize starch, or retrograded high-amylose maize starch induced different patterns of SCFAs in the portal blood of pigs during a 14-h test period (Martin et al. 2000). In addition, it was observed that acetate and butyrate molar ratios in the SCFA profile differed in vitro fermentation of eight native purified starches (RS2), which suggested that fermentation of RS was influenced by chemical composition and physical form of RS fermented (Giuberti et al. 2013). Similar results were also reported by Torres et al. (2013), who found that the concentration and composition of SCFAs differed after in vitro fermentation of five tropical legume grains. In summary, both the total SCFA production and the profile of each individual SCFA depended on several factors, including chemical composition, physical form, and availability of RS to ferment as well as the microbial population during fermentation. Different RS sources and types also might affect the site of fermentation in the large intestine (Giuberti et al. 2015). Slowly fermentable RS types in diets may provide substrates generating SCFAs in the more distal parts of the colon. In addition, the mixture of RS with different fermenting rates may provide substrates generating SCFAs in the whole colon.

44.4 Bioactivities (Animal Experiments)

Usually, starch is not considered as the biological active substance. However, RS cannot be digested in the small intestine and serve as a carbon source for bacterial fermentation in the large intestine. Therefore, RS is often considered as dietary fiber and displays important bioactivities. In addition, SCFAs are the major end products of gut bacterial metabolism of RS. A number of animal and human studies found that RS increased fecal excretion of SCFAs, specifically butyrate (Li 2010, 2018). Particularly, esterified or acylated forms of RS such as acetylated, butyrated, or propionylated RS4 confer specificity in the delivery of SCFAs, because these starches already carry specific SCFAs (Li 2018). These SCFAs loaded on the starches are only released in the large intestine, leaving the residual starch available for fermentation. Therefore, RS have the bioactivities resulting from SCFAs.

SCFAs are rapidly absorbed in the cecum and colon with only 5%–10% being excreted in the feces (Topping and Clifton 2001). Butyrate is the preferred energy source of colonocytes where oxidation of butyrate accounts for at least 60% of the cell's energy requirements, while other absorbed SCFAs enter the portal vein. Propionate is metabolized in the liver and thus is only present at low concentration in the periphery. Therefore, acetate is the most abundant SCFA in peripheral

circulation (Cummings et al. 1987). Furthermore, acetate can pass through the blood-brain barrier and decrease appetite via a central homeostatic mechanism (Koh et al. 2016). Among the SCFAs, butyrate particularly attracts considerable scientific interest due to its high efficiency in most bioactivities. For normal colon, butyrate promoted the integrity of the mucosal barrier, modulated the immune and inflammatory response, moderated fluid and electrolyte flux, and regulated colonic motility and cell growth and differentiation (Topping and Clifton 2001; Hamer et al. 2008). Importantly, animal experiments demonstrated that butyrate lowered colorectal oncogenesis, including reducing cell proliferation and inducing apoptosis of colorectal tumor cell lines (Perrin et al. 2001; Clarke et al. 2008; Le Leu et al. 2009). The mechanisms of lowering colorectal oncogenesis were complicated. Firstly, butyrate was able to inhibit histone deacetylases (HDACs) and thus affects gene expression (Gupta et al. 2006). Histone acetylation emerged as a central switch that regulated interconversion between permissive (via acetylation) and repressive chromatin structures (via deacetylation) (Koh et al. 2016). Histone acetylation is thought to increase accessibility of the transcriptional machinery to promote gene transcription. Acetyl groups are introduced to histone tails by histone acetyltransferases (HATs) and are removed by HDACs. As a result, HDAC inhibitors can be used for cancer therapy. Compared with normal colonocytes that consume butyrate, butyrate was accumulated threefold in nuclear extracts of cancer cells that consume glucose, resulting in higher concentrations of butyrate in cancerous epithelial cells (Donohoe et al. 2012). Thus, butyrate might act as an efficient HDAC inhibitor in cancerous cells rather than in normal colonocytes. Secondly, in vitro studies demonstrated that cell cycle arrest and apoptosis were induced in both a p53-dependent and p53-independent manners by butyrate at physiologically relevant concentrations (0.6–5 mmol/L) (Janson et al. 1997). In colorectal cancer cell lines, butyrate downregulated the expression of p53 mRNA and protein and also directly increased the expression of p53 target genes to induce cell cycle arrest (Gope and Gope 1993; Nakano et al. 1997). Thirdly, butyrate also altered gene expression through regulating the expression of micro-RNA rather than inhibiting HDAC (Fung et al. 2012).

In addition to being an antitumor agent, SCFAs have the anti-inflammatory effect in the large bowel. Rectal administration of either SCFA mixtures or butyrate alone was shown to effectively ameliorate the clinical symptoms of the disease in patients with distal ulcerative colitis (Luhrs et al. 2002; Scheppach et al. 1992; Breuer et al. 1991). The molecular mechanisms might be that butyrate reduces the expression of interleukin-8 and inhibits inducible NO synthase expression (Huang et al. 1997; Stempelj et al. 2007). In addition, it was reported that butyrate suppresses pro-inflammatory effectors due to inhibition of HDAC (Chang et al. 2014). Butyrate was also reported to modulate oxidative stress of healthy humans by increasing the level of glutathione in colonic mucosa (Hamer et al. 2009).

Another well-recognized general effect resulting from increased concentrations of SCFAs is to decrease the pH of the proximal colon. The pH in the colon can markedly affect composition of the colonic microbiota. It was reported that the final butyrate concentrations were significantly higher at pH 5.5 than at pH 6.5, which correlated with a change in the composition of the microbiota (Walker et al. 2005).

That is, the lowering of pH in the colon may promote butyrate production and improve populations of butyrate-producing bacteria. Moreover, the lowering of pH curtailed the growth of *Bacteroides* spp., propionate-producing bacteria. The inhibition effect of acidic pH is already recognized as an important factor to restrict the populations of certain pH-sensitive pathogens in the gut (Louis et al. 2007). On the other hand, the mildly acidic pH improved Ca^{2+} reabsorption from the colon (Abrams et al. 2005).

Therefore, the RS has the following health benefits, including the prebiotic effect, decreasing protein fermentation, keeping colon healthy, deducing postprandial glycemic response, inhibiting fat accumulation, reducing inflammation and oxidative stress, and improving mineral absorption.

44.4.1 The Prebiotic Effect

RS is proved to have the prebiotic effect by different methods from pure culture studies to animal experiments. Prebiotics are defined as nondigestible food ingredient that are beneficial to the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid 1995). Therefore, prebiotics improve host health. In vitro tests suggested that the environment was found to be dominated by the probiotic strains of *Bifidobacterium* and *Lactobacillus* in co-cultures of intestinal and probiotic bacteria in the presence of tartaric acid-modified dextrin (RS) (Barczynska et al. 2012). A RS-rich diet significantly increased the *Lactobacilli*, *Bifidobacteria*, and *Streptococci* populations, decreased the enterobacteria population, and altered the microbial enzyme metabolism in the colon of rats (Silvi et al. 1999). In another study, feeding high-amylose maize starch, one kind of RS, increased fecal *Bifidobacterium* numbers in mice (Wang et al. 2002). Pigs consuming high-amylose starch had higher fecal concentrations and excretion of *B. longum* than those consuming a conventional starch (Brown et al. 1997), which confirmed the prebiotic action of RS. In addition, it was reported that fructooligosaccharides (FOS) and RS raised fecal *Bifidobacterium* numbers by approximately equal amounts when they were fed separately (Brown et al. 1998). This result also confirmed the prebiotic effect of RS, since FOS is a well-known prebiotic. Interestingly, when FOS and RS were fed together, the increase of *Bifidobacteria* numbers exceeded the increase induced by individual, which suggested that the combination of FOS and RS resulted in a synergistic prebiotic effect. Their synergistic prebiotic effect was also confirmed by experiments in the rats (Rodriguez-Cabezas et al. 2010).

44.4.2 Decreasing Protein Fermentation

High-protein diet could result in an increase in protein fermentation in the large intestine, leading to an increased production of branched-chain fatty acids (BCFAs) and potentially detrimental metabolites, such as ammonia, amines, *N*-nitroso

compounds, phenols, thiols, and indoles (Cummings et al. 1979). It was reported that the high-RS (39 g/d) diet daily significantly increased fecal nitrogen and reduced excretion of fecal phenols, fecal concentrations of ammonia, and pH of human subjects (Birkett et al. 1996). However, daily output of urinary ammonia, urea, phenols, and total nitrogen did not significantly alter. These results suggested that RS might hinder protein fermentation, thus significantly attenuating accumulation of potentially harmful by-products from protein fermentation in the human colon. In addition, it was reported that a high-protein (25% casein) diet for 4 weeks led to a twofold increase in damage to colonocyte DNA in male Sprague-Dawley rats compared with a low-protein (15% casein) diet, which was associated with thinning of the colonic mucous barrier and increased levels of fecal *p*-cresol (Toden et al. 2005). However, addition of RS to the diet increased cecal SCFA pools and attenuated DNA damage, which confirmed that RS might inhibit protein fermentation and result in less genotoxic agents. Similarly, it was observed that feeding digestion-resistant potato protein increased the protein fermentation products in male Sprague-Dawley rats, which was reduced by adding RS to the diet (Le Leu et al. 2007). In another study, mice were fed 15% or 30% protein using casein or red meat or 30% protein with 10% high-amylose maize starch (equivalent to 5% RS2) (Winter et al. 2011). It was found that high protein diets increased promutagenic adducts (O^6-methyl-2-deoxyguanosine, O^6MeG) in the colon, while addition of 5% RS2 to the high protein diets lowered adduct formation, apoptosis, and fecal products of protein fermentation and increased production of butyrate. It was also found that RS inhibited protein fermentation by inocula from the large intestine of pigs using in vitro cultivation (He et al. 2017). In this study, fermentation patterns were analyzed during a 24-h incubation of cecal and colonic digesta with different RS contents using casein protein as the sole nitrogen source. The results showed that as the corn resistant starch levels increased, the SCFA concentration and cumulative gas production were significantly increased, while ammonia-nitrogen and BCFAs were decreased. The total bacteria, *Bifidobacterium* and *Lactobacillus*, were significantly increased with the increasing of the RS content after incubation. Therefore, it was concluded that addition of RS weakened the protein fermentation by altering microbial population. In summary, RS has the ability to decrease protein fermentation, which may be due to producing SCFAs and altering microbial population.

44.4.3 Keeping Colon Healthy

It is generally accepted that RS is beneficial to keep colon healthy, partially because RS increases fecal excretion of SCFAs, particularly butyrate. In addition, the prebiotic effect of RS may play a role in colon health. Colon health involves maintaining normal function of the colon. Healthy colon has regular bowel movement once a day or more frequently with the feces being relatively soft but non-diarrhetic. Colon health can be reflected in the measurement of laxation. It was observed that the RS supplement increased the fecal bulk by 22 g/day compared with the low-fiber control, while the wheat bran supplement increased fecal bulk 96 g/day (Jenkins

et al. 1998). This result suggested that RS was able to increase the fecal weight, but its ability was much weaker than wheat bran. However, RS and wheat bran significantly increased fecal weight in another study and did not differ from each other when 14 subjects were given 25 g RS (PROMITOR^{TM}, Tate & Lyle Americas, Decatur, IL, USA) or wheat bran fiber per day for 14 days (Maki et al. 2009). The different result might be due to the difference of the RS type used.

The colon health also involves prevention of colon diseases. Several studies have investigated the effect of RS on colon cancer prevention by animal experiments. In most cases, RS has been fed in diets combining treatment by a chemical carcinogen to test its effect on preventing colon cancer. For instance, Sprague-Dawley rats were fed diets containing no RS or digestion-resistant potato protein (PP), 10% raw high-amylose corn starch (HAS, source of RS2), 15% PP, or 10% HAS and 15% PP for 4 weeks prior to treatment by azoxymethane (AOM), and colon cancers were assessed 30 weeks after AOM treatment (Le Leu et al. 2007). The RS inhibited colon tumor development and increased SCFAs including butyrate in the distal colon. In addition, the RS lowered production of potentially toxic protein fermentation products. These suggested that RS not only protected against intestinal tumorigenesis but also ameliorated the tumor-enhancing effects of feeding indigestible protein. They later confirmed that feeding the same RS2 protected against AOM-induced colon carcinogenesis and favorably influenced the colonic luminal environment (Le Leu et al. 2014). In the experiments, male Sprague-Dawley rats were provided with one of three diets, control (without RS), 10% HAS, and 20% HAS for 4 weeks, and then injected with AOM (15 mg/kg) during the 5th and 6th week. Data demonstrated that feeding RS significantly reduced the incidence and multiplicity of adenocarcinomas in the colon compared to the control diet. Both doses of HAS resulted in similar protection against colon tumorigenesis. Similarly, Yuan et al. (2017b) found that RS reduced the numbers of aberrant crypt foci (ACF) and aberrant crypts of mice with AOM-induced early colon cancer. In another study, the RS completely prevented the development of tumors in Sprague-Dawley rats, compared to rats fed control starch, when rates were fed RS for 20 weeks following treatment by 1, 2-dimethylhydrazine (Bauer-Marinovic et al. 2006). It was found that this effect was mediated by enhanced apoptosis of damaged cells accompanied by changes in parameters of dedifferentiation in colonic mucosa. Nakanishi et al. (2003) investigated the inhibitory effects of RS2 and *C. butyricum* strain MIYAIRI 588 (CBM588) on AOM-induced ACF formation in rats. Administering only CBM588 spores increased the concentration of butyrate in the cecum, but did not decrease in the number of ACF. Administering only RS2 or RS2 and CBM588 spores decreased the number of ACF. In these two groups, the concentrations of acetate and propionate in intestinal contents were significantly increased, but the concentration of butyrate did not change. However, the β-glucuronidase activity level of colonic contents was significantly decreased in the two groups of rats fed RS2. These results showed that RS and CBM588 changed metabolism of colonic microbiota and decreased the β-glucuronidase activity, which played a role in the inhibition of ACF formation in the rat colon. In summary, RS can help to prevent colon cancer, but the mechanism is complicated.

RS has been proved to be able to prevent or reduce inflammatory bowel diseases. For rats with colitis induced by trinitrobenzenesulphonate (TNBS), RS accelerated healing via prebiotic and butyrate effects (Jacobasch et al. 1999). Moreau et al. (2003) compared FOS and RS in healing colonic inflammation of dextran sulfate sodium (DSS)-induced colitis rat model and found that intake of RS significantly improved colon histopathology scores compared to control and FOS and also increased SCFA concentrations in cecal contents. Long-term intake of RS showed increased the butyrate content of pigs, reduced damage to colonocytes, improved mucosal integrity, and reduced colonic and systemic immune reactivity, which suggested that RS might help pigs to respond to intestinal inflammation better (Nofrarias et al. 2007).

In addition, long-term intake of RS diet increased crude protein and mucin contents and upregulated the expression of mucin genes MUC4, MUC5AC, and MUC12 in the colons of pigs, suggesting the potential of long-term intake of RS diets to improve colon health by increasing mucin secretion and reducing the harmful fermentation of protein (Zhou et al. 2017).

44.4.4 Reducing Postprandial Glycemic Response

Blood glucose concentration control after consuming a meal is primarily determined by the rate of appearance of glucose from the gastrointestinal tract and its clearance from the circulation (Robertson 2012). Many factors affect blood glucose concentration, but insulin is the most important one. Insulin controls the blood glucose concentration via a classical feedback loop. A rise of blood glucose concentration stimulates the secretion of insulin from β cells of the pancreas, and the resulting insulin stimulates muscle and adipose tissue to increase glucose uptake, thus declining blood glucose concentration. Insulin secretion indicates the ability of a rise in plasma glucose to stimulate insulin secretion, and insulin sensitivity indicates the ability of insulin to stimulate glucose uptake from the blood. Normally, insulin secretion maintains blood glucose concentration within a narrow range. However, insulin resistance, defects in insulin secretion, or both impair both fasting and postprandial glucose regulation of individuals, leading to a rise in the blood glucose concentration. As a result, diabetes may occur. Diabetes has serious complications, including heart disease, kidney disease, eye disease, cerebrovascular disease, and nerve damage. Reducing blood glucose levels can prevent and delay the onset of these complications or reduce the severity for patients with diabetes. It is also known that reducing postprandial glycemic response is beneficial to prevent type 2 diabetes for people with high blood glucose concentration or obesity. Consequently, reducing postprandial glycemic response is important for people with high blood glucose, particularly patients with diabetes and prediabetes.

The ability of RS to decrease blood glucose was confirmed in normal rice model. Male Wistar rats were divided into four groups and fed wheat bread, RS-wheat bread, maize bread, and RS-maize bread (Brites et al. 2011). It was found that the RS-wheat bread group significantly reduced feed intake, fecal pH, postprandial

blood glucose response, and total cholesterol. The RS-maize group significantly reduced body weight gain, fecal pH, and total cholesterol levels; but only a reduction in fasting level was observed in the glycemic response. These results confirmed the effect of RS on glycemic response and suggested that the magnitude of the effect of RS on glycemic response depended on other components of diets. Diabetes rice model was also used to confirm the hypoglycemic effect of RS. In a study, RS2 (Hi-maize starch containing 60% amylose) improved glucose tolerance and reduced body fat in the Goto-Kakizaki rat, a nonobese model of type 2 diabetes (Shen et al. 2011). Specifically, feeding RS greatly improved pancreatic β-cell mass, insulin sensitivity, pancreatic insulin content, total GLP-1 (glucagon-like peptide-1) levels, cecal SCFA concentrations, and butyrate-producing bacteria in cecal contents. In another study, the hypoglycemic effect of low, medium, and high doses of RS2 (Hi-maize 260;100, 150, and 200 g/kg) for 28 days was evaluated, and the potential mechanism of this effect was explored in type 2 diabetic rats treated with high-glucose/high-fat diet and low-dose streptozotocin (STZ) (Sun et al. 2018). Feeding RS induced better regulation of oral glucose tolerance test, insulin, glucose metabolism, lipid in plasma and liver, fructosamine, and pancreatic damage in diabetic rats. Interestingly, the medium-dose RS treatment had the best hypoglycemic activity. These results suggested that RS regulated the blood glucose levels of diabetic rats through altering the expression levels of the genes related to glucose metabolism and ameliorating pancreatic dysfunction.

In addition, studies have been done on the effect of different RS sources on blood glucose in humans. For instance, when patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or newly diagnosed type 2 diabetes ($n = 90$) were randomly assigned to either a group ingesting rice containing 6.51 g RS daily or a control rice group for 4 weeks, the diet containing rice with RS reduced fasting insulin and insulin resistance, postprandial glucose and insulin levels at 30 min, and glucose and insulin areas under the response curve after the standard meal (Kwak et al. 2012). In another study, the test was conducted in 20 subjects (9 men and 11 women with a mean age of 50.5 years) using the crossover method, with a single ingestion of either bread containing RS3 (tapioca maltodextrins were debranched and then retrograded) or the placebo (Yamada et al. 2014). Postprandial increases in blood glucose and blood insulin were significantly decreased in subjects with the blood glucose level before ingestion $\geq$111 mg/dl who took the test food compared with the placebo group. Recently, Mah et al. (2018) designed one experiment in which 21 healthy adults consumed a baked breakfast bar containing tapioca-based RS4 (Actistar 75330; Cargill, Inc.) or a macronutrient-matched control bar and found that compared with the control food, consumption of the RS4 food decreased the incremental area under the curves from 0 to 120 min ($iAUC_{0–120\ min}$) for postprandial capillary glucose and $iAUC_{0–120\ min}$ of insulin by 22% and 37%, respectively. Similarly, it was found that adding a practical dose of RS4 (RSVERSAFIBE™ 2470) in muffin significantly reduced postprandial glucose and insulin responses in healthy adults, which was reflected by reduction in $iAUC_{0–120\ min}$ of glucose, maximum glucose concentration, and $iAUC_{0–120\ min}$ of insulin (Stewart and Zimmer 2018).

In summary, RS consumption has been proved to be able to improve glycemic control in both animal and human studies. Blood glucose level is affected by several factors, including absorption, clearance, and release from internal organs (Wong and Louie 2017). Due to escaping digestion of small intestine, RS absolutely lowers glycaemia when it replaces the available carbohydrate portion of a meal. This effect is reflected by the glycemic index (GI), which indicates glycemic response of different food items upon consumption. RS does not induce indicates glycemic response and belongs to low-GI food. Importantly, RS is able to decrease postprandial glycemic response when the available carbohydrate portion of the diet is not reduced, which suggests RS decreases postprandial glycemic response by other mechanisms. The mechanisms behind are complicated. Particularly, RS is able to improve insulin sensitivity and β-cell function (insulin secretion). Improvements in muscular and hepatic glucose handling may be another mechanism. For instance, it was found that the RS-treated mice expressed more G-protein coupled receptors (GPR) 41 and 43 than with normal rice-treated mice (Yuan et al. 2017a). The GPR 41 and GPR 43, which are SCFA receptors, have been found to lead to an increase in glucose uptake and glycogen storage at muscle tissues (Canfora et al. 2015). In addition, acetate was reported to reduce hyperglycemia in diabetic KK-A(y) mice through activating 5′-AMP-activated protein kinase (AMPK) in the liver, since AMPK played an important role in activating glucose and fatty acid uptake and oxidation (Sakakibara et al. 2006). Another possible mechanism where RS consumption may influence on blood glucose control is that RS upregulates gut hormones, including GLP-1 and peptide YY (PYY) (Zhou et al. 2008; Shen et al. 2011). These two hormones are naturally secreted in response to meal ingestion, but they are rapidly degraded after endogenous secretion or exogenous injection (Zhou et al. 2008). GLP-1, a potent incretin by the enteroendocrine L cells of the distal intestine, is shown to possess multiple effects on glucose metabolism, such as promoting pancreatic β-cell mass, stimulating glucose-dependent insulin secretion, and inhibiting glucagon secretion (Shen et al. 2011). PYY, a 36-amino-acid peptide hormone that is cosecreted from intestinal L cells with GLP-1, is initially found to inhibit appetite, thus lowering energy intake (Manning and Batterham 2014).

44.4.4.1 Inhibition of Fat Accumulation

RS, as one kind of the dietary fiber, was able to lower plasma cholesterol and triglyceride concentrations and reduce fat storage (Higgins 2004; Nugent 2005). Total cholesterol, low-density and very low-density lipoprotein cholesterol, and triglycerides were significantly lowered in serum of hamsters fed on the diet containing extruded cassava starch and RS by 17.87%, 62.92%, and 9.17%, respectively, as compared with the diet of cassava starch without added RS (Martinez-Flores et al. 2004). In another study, the effects of RS and cellulose on blood and liver lipids in hamster were compared, and it was observed that RS and cellulose decreased serum cholesterol level by 16.2% and 13.5%, respectively (Ranhotra et al. 1996a). Recently, the effects of RS on postprandial increases in blood triglyceride levels were investigated in rats using oral fat tolerance/loading tests (Matsuda et al. 2016). After administration of lipid meals, feeding RS evidently declined increases

in serum triglycerides levels of rats. In addition, rats fed corn oil containing 500 mg/mL RS has much greater fecal lipid volumes and wet weights following lipid meals than rats fed only corn oil, which confirmed that fat absorption was inhibited by RS. The RS type also affected its anti-obesity effect. For instance, mice fed the RS4 diet had lower body weight and visceral fat weight than those fed either the unmodified starch or RS2 diet, when male C57BL/6 J mice were fed on a high-fat diet containing unmodified starch, hydroxypropylated distarch phosphate (RS4) or RS2 (high-amylose starch) for 24 weeks (Shimotoyodome et al. 2010). In addition, mice fed the RS4 diet had a higher hepatic fatty acid oxidation capacity and related gene expression and lower blood insulin than the other two groups. When given with fat (trioleate) by gavage, dietary supplementation with RS4 stimulated a lower postprandial glucose-dependent insulinotropic polypeptide (GIP; incretin) response than RS2. The GIP could decrease fat utilization in high-fat diet-fed mice. These results suggested that RS4 attenuated high-fat diet-induced obesity more effectively than RS2, which may be due to lower postprandial GIP and increased fat catabolism in the liver.

As described above, the anti-obesity effect was usually accompanied by the hypoglycemic effect. Several reports have suggested that these effects are partially ascribed to increased SCFAs production in the bowel (Sakakibara et al. 2006; Yamashita et al. 2014; Gao et al. 2009; Arora et al. 2011; Lin et al. 2012). When acetate was orally injected to obesity-linked type 2 diabetic Otsuka Long-Evans Tokushima fatty rats at the dose of 5.2 mg/kg BW, acetate markedly reduced in lipid accumulation in the adipose tissue, protected against accumulation of fat in the liver, and improved glucose tolerance (Yamashita et al. 2014). In another study, supplementation of butyrate enhanced adaptive thermogenesis and fatty acid oxidation, and there is an increased mitochondria function and biogenesis in the skeletal muscle and brown fat when butyrate was administrated in dietary obese C57BL/6 J mice through diet supplementation at 5% w/w in the high-fat diet (Gao et al. 2009). Supplementation of butyrate prevented obesity in C57BL/6 J mice, while fasting blood glucose and insulin tolerance were observed in the mice fed on the high-fat diet. It had been shown that propionate inhibited hepatic cholesterol synthesis in humans and also played a role in regulating food intake in non-ruminants (Arora et al. 2011). In addition, a later study demonstrated that butyrate and propionate suppressed food intake, protected against high-fat diet-induced weight gain and glucose intolerance, and stimulated gut hormone secretion predominantly via free fatty acid receptors 3-independent mechanisms (Lin et al. 2012).

44.4.4.2 Reducing Inflammation and Oxidative Stress

Systemic inflammation and oxidative stress play an important role in the pathogenesis of cardiovascular diseases and complications of chronic kidney disease (Tayebi Khosroshahi et al. 2018). Therefore, it is important to reduce inflammation and oxidative stress. Yuan et al. (2017a) found that after diabetic mice were treated with normal rice, normal rice with RS, or normal rice with RS and Se for 4 weeks, supplementing with RS lowered levels of serum C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nuclear factor-k-gene binding

(NF-κB), and leptin (LEP) and increased adiponutrin (ADPN) levels. In addition, Se and RS decreased CRP, IL-6, and NF-κB levels much more than RS. These results indicated that RS reduced inflammation and Se and RS might have synergistic effects on chronic inflammation. RS was also proved to reduce inflammation and oxidative stress in humans. For instance, it was found that 4-week dietary treatment with RS also reduced oxidative stress of patients with IFG, IGT, or newly diagnosed type 2 diabetes when this treatment reduced their blood glucose level (Kwak et al. 2012). In another study, 46 stable hemodialysis patients randomly consumed biscuits containing 20 g/day during the first 4 weeks and 25 g/day in the following 4 weeks of either RS2 or wheat flour (Tayebi Khosroshahi et al. 2018). RS2 significantly declined serum levels of TNF-a, IL-6, and malondialdehyde compared with the placebo. In addition, serum urea and creatinine concentrations were significantly decreased, and severity of constipation was improved in RS2-treated patients. These results suggested that administration of RS2 for 8 weeks significantly reduced levels of inflammatory and oxidative markers in hemodialysis patients. Similar results were obtained by Esgalhado et al. (2018). This study evaluated 31 hemodialysis patients assigned to either RS (16 g Hi-maize 260) or placebo (manioc flour) supplementation, which they received for 4 weeks on alternate days through cookies on dialysis days and powder in a sachet on non-dialysis days. It was found that the RS group had lower IL-6, thiobarbituric acid reactive substances plasma (TBARS), and indoxyl sulfate plasma levels, while no significant differences were observed in the placebo group. These reports confirmed that RS had the ability to reduce inflammation and oxidative stress.

44.4.4.3 Improving Mineral Absorption in Large Intestine

As mentioned above, SCFAs produced from fermentation of RS2 decreased the pH, which was beneficial to mineral absorption. Therefore, RS might improve mineral absorption, which was confirmed by several studies. For example, Lopez et al. (2000) investigated the effects of a natural source of phytic acid, wheat bran, in the presence or in the absence of RS on the assimilation of minerals (Ca, Mg, and P) and trace elements (Fe, Mn, Zn, and Cu) in rats adapted to semi-purified diets and found that absorption of Ca, Mg, and P in the cecal was 3.5-fold higher in the RS groups than in the control groups due to the hypertrophy of the cecal wall, low luminal pH, and improved concentrations of soluble minerals. Moreover, the apparent retention of all the above minerals was significantly enhanced by RS ingestion. The disappearance of phytic acid was twofold higher in rats fed the RS diet than those fed the control diet. Thus, it was concluded that the addition of RS into wheat bran diet allowed a greater mineral absorption by increasing the SCFA production and breaking down phytic acid in the large intestine. Likewise, Yonekura et al. (2003) found that RS2 restored Zn and Mg bioavailability suppressed by phytic acid in rats, which was due to that cecal fermentation of RS2 increased SCFA and succinate concentrations and reduced cecal pH. In another study, the effects of RS2 and RS3 on mineral absorption, including Ca, Mg, and Fe, were investigated (Zeng et al. 2017). Specifically, BALB/c male mice were fed five different diets: Diet 1 containing no RS; Diet 2, 3, and 4 containing 5, 10, or 15 g RS3/100 g diet; and

Diet 5 containing 15 g RS2/100 g diet. Data demonstrated that the apparent absorption of Ca was significantly greater in mice fed medium and high levels of RS3, as well as RS2, than in those fed the basal and the low-level RS3 diets. The mice fed high levels of RS3 displayed the greatest apparent absorption of Ca. Similar results were obtained when the effect of RS on Mg absorption was studied. In addition, mice fed on RS3 and RS2 exhibited greater apparent absorption of Fe, and the apparent absorption of Fe was enhanced as the RS3 dose increased. These results might be related to SCFAs by the intestinal microbial fermentation of RS. In addition, it was reported that bread with RS4 and garlic showed a prebiotic effect and increased Ca bioavailability and deposition in bones in male weaning Wistar rats, compared with wheat bread (Weisstaub et al. 2018). However, Schulz et al. (1993) found that apparent absorption of Ca and Mg in rats was improved by RS2 (uncooked high-amylose starch granules) but not by RS3 (cooked and cooled high-amylose starch). Compared with cooked normal starch, RS2 significantly lowered the ileal pH, while RS3 raised it. Cecal pH was lowered by the two kinds of RS. Ca concentrations in the liquid ileal contents were improved by RS2 but were significantly lowered by RS3 relative to control starch. Ma and Ca concentrations in liquid cecal contents were raised by RS2, but RS3 did not change them. These results suggested that RS3 might be not fermented in the ileum since the pH was not decreased. Thus, it was inferred that RS3 might be fermented at a very slow rate, resulting in no increase in Ca and Mg absorption. To summarize, consumption of RS may improve mineral absorption in large intestine. However, this effect may be removed once it is fermented too slowly.

44.5 Function in Human (Human Studies)

The function in human of starch mainly results from metabolism of glucose digested from starch. Glucose, an essential nutrient of living organisms, not only provides the potential energy but also acts as a precursor for metabolic intermediates in biosynthetic pathways (Miao et al. 2015a). Humans require a reliable source of glycemic carbohydrate to support the normal functions of our brain, red blood cells, muscles, kidney medulla, and reproductive tissues. Here, we summarized the function in humans of starch from mental and physical performance like Hendrich (2018).

44.5.1 Function in Mental Performance

Glucose is the main energy source for brain of humans, although brain has the ability to utilize other fuel molecules, such as ketone bodies (Nirmalan and Nirmalan 2017). The adult human brain accounts for only around 2% of body mass but around 20% of whole body resting energy expenditure (Wang et al. 2014; Mergenthaler et al. 2013). Thus, the brain is the main consumer of glucose, approximately consuming 5.6 mg glucose per 100 g human brain tissue per minute. In human newborns, the brain weighs approximately 11% of body weight but consumes >50% of energy (Wang

et al. 2014). Up to the age of 3, when the brain size rapidly increases, it is recommended that at least one-third of dietary energy should be supplied from carbohydrates (Bier et al. 1997). Neuronal computation and information processing, such as the generation of action potentials and postsynaptic potentials generated after synaptic events, consume the largest proportion of energy in the brain (Harris et al. 2012). In addition to providing primary energy of the brain, glucose metabolism plays an important role in physiological brain function through the foundation of neuronal and non-neuronal cellular maintenance and generating neurotransmitters (Mergenthaler et al. 2013). For instance, it seems that astrocytic glycogen is very important for learning, since glycogen selectively supplies carbon and supports de novo synthesis of transmitter glutamate by combined pyruvate dehydrogenation and carboxylation in astrocytes (Hertz and Gibbs 2009). Furthermore, breakdown of astrocytic glycogen and release of lactate from glycolysis are essential for forming long-term memory and for maintaining the long-term potentiation of synaptic strength elicited in vivo (Suzuki et al. 2011). Thus, the brain increases consumption of glucose upon activation (Sokoloff 1999).

In contrast to other tissues, the brain of humans at birth is very immature and undergoes substantial quantitative and qualitative changes during postnatal development, which need substance foundation and energy. Therefore, it seems that glucose metabolism is important for human brain evolution. In addition, abnormal glucose metabolism declines cognition. A meta-analysis of several hundred children with type 1 diabetes (T1D) showed that individuals with T1D had lower scores at overall IQ, executive function, and motor speed than control children without T1D (Tonoli et al. 2014). The same cognitive impairments were seen in adults with T1D, and memory of these adults was also impaired compared with control adults without T1D. In addition, it seemed that the longer the duration of T1D, the more cognition may be impaired. On the other hand, glucose-enhanced cognitive performance is consistently observed in populations who usually have poorer memories and glucose regulation, such as healthy elderly subjects and patients with Alzheimer's disease (Greenwood 2003). This result suggests that glucose can reverse or mask the memory deficits observed in those with poor gluco-regulatory status and/or underlying memory deficits (Greenwood 2003).

44.5.2 Function in Physical Performance

Physical activity requires energy for muscle contraction. As stated above, free glucose from muscle glycogen is not released into the circulation, which indicates that glucose is of great importance as a muscle fuel. Particularly, anaerobic metabolism of glucose can rapidly provide energy during very high-intensity physical activity such as a sprint. It is generally recognized that a decrease in carbohydrate availability can result in fatigue during prolonged exercise in humans (El-Sayed et al. 1997). During prolonged exercise, blood glucose and muscular glycogen are the two major sources of carbohydrate utilization by the active muscles. During exercise, the energy from blood sugar is limited for normal humans. Therefore, long-

term exercise performance may mainly depend on the muscle glycogen stores (Hendrich 2018). It has been shown that administration of glucose or other carbohydrates before or during exercise postpones fatigue, conserves muscle glycogen, and improves performance. Thus, replenishing enough muscle glycogen stores is the main concern for athletes who need extreme endurance.

Particularly, effects of starches on exercise of people with diabetes have been studied. A previous study compared metabolic responses and fuel use of participants with T1D during sub-maximal and high-intensity performance running following pre-exercise ingestion of 0.6 g/kg body mass waxy barley starch or dextrose (Gray et al. 2015). Interestingly, T1D individuals consuming waxy barley starch had a greater carbohydrate oxidation rate at rest and displayed an improved performance at the latter stages of a high-intensity run test, although waxy barley starch and dextrose led to similar hyperglycemic responses. In another study, people with type 2 diabetes (T2D) were fed on a vegetarian diet with 60% carbohydrate or a conventional diet with 50% carbohydrate for 12 weeks at 500 kcal restriction of daily energy requirement and had personalized daily exercise (Veleba et al. 2016). It was observed that the vegetarian diet improved fitness while the conventional diet did not. This suggests that a vegetarian diet higher in complex carbohydrates might benefit people with T2D, as improved physical fitness may help people persist with increased physical activity. Therefore, much work is required to determine what starch types optimize exercise performance for different people and the underlying mechanisms.

44.6 Safety

According to the long-term eating habits of humans, native starches are generally safe and well tolerated. They have little chance for adverse effects except those effects associated with long-term overconsumption (Hendrich 2018). The dose makes the poison, which is a central tenet of toxicology. Therefore, even glucose, the digestion product of safe starches, may be toxic to humans. As stated above, normal people can decline the postprandial blood glucose to the normal level. But some people suffer from hyperglycemia. Therefore, glucose toxicity occurs, and it usually refers to damaging effects of high blood glucose concentrations on body tissues and regulatory processes through several mechanisms (Brownlee 2005). First, the mechanisms involve the polyol pathway, particularly aldose reductase. Normally, aldose reductase reduces toxic aldehydes in the cell to inactive alcohols. However, when the glucose concentration in the cell becomes too high, aldose reductase also reduces glucose to sorbitol. During this process, NADPH is consumed as the cofactor of aldose reductase. But NADPH is also the essential cofactor for regenerating reduced glutathione. Due to the decreasing amount of reduced glutathione, the polyol pathway increases susceptibility to intracellular oxidative stress. Secondly, glucose can interact with free amines in body proteins and form advanced glycation end products (AGEs). AGEs can directly induce cross-linking of long-lived proteins such as collagen (Goh and Cooper 2008). Thus, vascular stiffness is promoted. In addition, AGEs can enhance oxidative stress and elaborate key

proinflammatory and prosclerotic cytokines via interaction with certain receptors (Wautier et al. 2017; Goh and Cooper 2008). Thirdly, the mechanisms involve the protein kinase C (PKC) pathway. In this pathway, hyperglycemia inside the cell increases the synthesis of diacylglycerol, which is a critical activating cofactor for the classic isoforms of protein kinase C, β, δ, and α. Once PKC is activated by intracellular hyperglycemia. It has a variety of effects on gene expression. The pathological effects that may result from activation of PKC include blood flow abnormalities, vascular permeability angiogenesis, capillary occlusion, vascular occlusion, proinflammatory gene expression, etc. Fourthly, some of fructose-6-P resulting from glucose gets diverted into a signaling pathway in which GFAT (glutamine:fructose-6-P amidotransferase) converts the fructose-6-P to glucosamine-6-P and finally to UDP (uridine diphosphate) *N*-acetylglucosamine. Subsequently, the *N*-acetylglucosamine is transferred onto serine and threonine residues of transcription factors, and overmodification by this glucosamine often results in pathologic changes in gene expression. A unified mechanism is that hyperglycemia increases superoxide production and oxidative stress. Therefore, rapidly digestible dietary starches may contribute to such pathologies for those who suffer from hyperglycemia.

Many chemically modified starches made for food use are safe because these modified starches are allowed to contain only small amounts of substituent groups (Singh et al. 2007). When starch octenyl succinates are produced for application in foods, the amount of OSA is limited to 3% based on the dry starch weight (the degree of substitution <0.0231) (Altuna et al. 2018). The maximum permitted amount of substitution groups for starch phosphates, starch acetates, and hydroxypropylated starches are 0.4%, 2.5%, and 10%, respectively (Chen et al. 2018). Similarly, cross-linked food starches are allowed to contain one substituent cross-linking group per 1000 or more anhydroglucose (Singh et al. 2007).

44.7 Products in Market

A vast range of native starches are already at the market, including maize starch, cassava starch, potato starch, wheat starch, rice starch, waxy starches, etc. Physicochemical properties of starches from different sources differ significantly. Worldwide, maize (82%), wheat (8%), potatoes (5%), and cassava (5%) are the main sources of starch (Corre et al. 2010). In the food industry, modified starches mainly include pregelatinized starch, maltodextrin, oxidized starch, hydroxypropyl starch, starch octenyl succinate, starch acetate, starch phosphates, and cross-linked starch.

In addition to natural food sources of RS stated above, increasing commercially manufactured forms of RS are available. Commercial sources of RS2, amylomaize VII (Cerestar Inc., Hammond, IN, USA), Hi-maize 260 (National Starch & Chemical Co, Bridgewater, N.J., USA), and Hylon VII (National Starch & Chemical Co, Bridgewater, NJ) are now available (Ranhotra et al. 1996b; Martínez et al. 2010; Hylla et al. 1998). CrystaLean (Opta Food Ingredients, Inc.), NOVELOSE 330® (National Starch & Chemical Co), Hi-maize 330 (National Starch & Chemical Co),

and Promitor Resistant Starch 60 (Tate & Lyle) are examples of commercially developed RS3 which are derived from high-amylose maize starch (Nugent 2005; Maningat and Seib 2013). To promote formation of RS3, the starch was first hydrolyzed by a debranching enzyme to increase the amount of linear chains, followed by crystallization of the linear chains to (Maningat and Seib 2013). One commercially available example of RS3 produced in this way is Actistar™ (Cargill Inc.), which is produced by debranching and crystallization of tapioca maltodextrin (US6043229) (Kettlitz et al. 2000). To further promote crystallization of starch, Tate & Lyle first treated starch with a glucanotransferase to elongate the external chains of amylopectin, followed by debranching and then crystallization of the linear chains to form RS3 (US7674897B2) (Norman et al. 2010). RS4 products are also commercially available. For example, Fibersym RW (MGP Ingredients, Atchison, Kansas, USA) is a phosphorylated cross-linked wheat starch (Woo and Seib 2002). Actistar 75330 is a phosphorylated RS4 derived from tapioca that is commercially available from Cargill, Inc. (Mah et al. 2018). VERSAFIBE™ 2470 is a newly developed RS4 by Ingredion Incorporated (Bridgewater, NJ) that is derived from high-amylose maize starch modified by acid hydrolysis and heat treatment (Stewart and Zimmer 2018). Dextrinization of starch can lead to the formation of potentially indigestible linkages. Thus, dextrinization is used by several companies to create indigestible dietary fiber ingredients that can be classified as RS4 (Maningat and Seib 2013). Nutriose® soluble fibers are food dextrins derived from wheat or maize starch (US5620871) (Caboche 1997), which are marketed by Roquette (Roquette Frères). Fibersol 2 is a resistant maltodextrin reported in the USA patent (US5358729) (Ohkuma et al. 1995) and is produced by dextrinization of starch followed by heating at 120 to 200 °C. This RS is produced and marketed by a joint venture between Archer Daniels Midland Company and Matsutani (Matsutani LLC). Tate & Lyle's Promitor™ Soluble Corn Fiber ingredients are characterized by a higher concentration of nonlinear saccharide oligomers, which result from treating starch by cooking, hydrolysis, enzyme depolymerization, fractionation, isomerization, etc. (US7608436) (Harrison et al. 2009).

44.8 Perspective

Glucose is an important fuel for humans, particularly brain, muscles, red cells, etc., and starches in human diets are the main providers of glucose. According to the previous results, dietary starch plays an important role in development of the human brain, and tightly regulating blood glucose may be better for cognition. Therefore, it is meaningful to discover, design, and further develop diets and starches that benefit brain development and cognition. Therefore, recommendations for human dietary patterns must include the dietary starch content and type (Hendrich 2018). In addition, there is an additional opportunity to design starches that benefit exercise performance. In summary, starch deserves more attention as a foundation of a healthy diet for cognitively sound and physically active humans (Hendrich 2018). That is, starch ingestion must be optimized and individualized to meet special needs of humans.

On the other hand, effects of RS on gut microbiota and effects of microbiota on RS metabolism are still the focus of scientists. It is possible that different forms of RS are accessible by different groups of colonic microorganisms. Thus, different types of RS may promote different groups of colonic bacteria. This could result in selective effects of RS intake upon the species composition of the colonic microbiota, as well as differential effects on gut metabolism. It highlights the need to consider both primary degraders of RS and specific more-downstream-acting bacterial groups in order to achieve desired intervention outcomes. The gained insights will assist the design of personalized treatment strategies based on an individual's microbiota (Vital et al. 2018). To better interpret the relationship between gut microbiota and RS, new techniques are required to analyze gut microbiota. In addition, new and advanced analytical methods for RS are required, because the analysis of RS still greatly depends on methods developed earlier for dietary fiber (Birt et al. 2013). On the other hand, the previous studies suggest that RS structure determines its rate and site of fermentation in the large intestine. The characteristics of dietary starch, including particle structure, crystallinity, branching, association with other polymers, retrogradation, and modification, are known to affect its digestibility in the small intestine (Miao et al. 2015a, 2018). It is inferred that these characteristics may also affect the rate at which RS is fermented in the colon. Rapid fermentation of RS may lead to complete fermentation in the proximal colon, whereas slower rates support fermentation in more distal regions, with the possibility of incomplete colonic fermentation overall. Fermentation kinetics, combined with information about the transit time, can give an indication about where ingredients are fermented and could therefore be used to select RS sources eliciting fermentation in specific places of the guts. Therefore, it seems possible to control the fermentation rate and site. Accordingly, different RS types in diets may provide enough substrates generating SCFAs in the more distal parts of the colon with more possible reduction in protein fermentation. Recently, imbalances in human gut microbiota are considered to be related to various noncommunicable diseases, such as colon cancer, type 2 diabetes, and obesity. Thus, RS have the potential to prevent these diseases, since RS can modulate gut microbiota. However, considerable research is required to identify the potential effectiveness of RS in preventing or even cure human diseases. Particularly, despite the tremendous diversity of RS in plants and commercial RS products, very few of them have been studied. In conclusion, future integrative research is needed to expand the potential uses of RS in health promotion of humans.

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