Mallappa Kumara Swamy *Editor*

Plant-derived Bioactives

Chemistry and Mode of Action

Springer

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Dedicated to My
Parents, Family Members,
and Teachers
Who Taught Life Lessons

Foreword

Plant-derived bioactive compounds elicit several positive health benefits in humans. Plants and their extracts/preparations have been utilized as traditional medications, since ancient times, throughout the world. In this regard, phytotherapy practices, such as Ayurveda, Unani, Siddha, and the Traditional Chinese medicines, exemplify essentially a vital part of our traditional information and heritage. Different plant organs, including leaves, roots, stem bark, fruits, vegetables, seeds, etc., are being used in these traditional medicare practices. Plants possess bioactive metabolites with a high chemodiversity and offer good candidates in designing novel drugs against various ailments. Also, these phytocompounds are considered as the most suitable chemical compounds, and are safe to be used as alternative medicines. Hence, therapeutic functions of plant-derived bioactives still remain to be the major topic of several researches. In particular, the traditional uses of plant parts in medicinal preparations have instigated the exploration of such plants for different metabolites to be used as drugs or leads in the drug discovery. In this direction, several medicinal and aromatic plants, including *Podophyllum peltatum, Catharanthus roseus, Camptotheca acuminata, Tinospora cordifolia, Coleus forskohlii, Centella asiatica,* and many others have become the source of bioactive principles. Several bioactive molecules, such as gymnemic acids, orientin, coumarins, ginsenosides, chromenes, capsaicin, vinblastine, vincristine, camptothecin, and podophyllotoxins have been extracted from different plant species. Several of these bioactives have shown significant therapeutic benefits, and some of them are being used in the treatment of cancers, diabetes, cardiovascular diseases, and several other human diseases. However, biological functions of many such bioactive molecules are yet to be understood in detail. Moreover, the nature constitutes numerous plant species that are yet to be explored in detail for bioactive metabolites. Hence, a regulated exploration of plant biodiversity and a better understanding on the chemistry and mode of actions of plant-derived metabolites could be very useful in designing drugs/lead molecules for an effective treatment of ever-challenging diseases, including microbial infections. Further, plant metabolite's mechanisms of action allow one to design effective therapeutic agents and to make use of the target-specific drug delivery and therapy, which also involves nanotechnology.

This book volume, *Plant-derived Bioactives: Chemistry and Mode of Action*, Volume 1, published by "Springer" consists of 26 chapters covering diverse topics related to plant secondary metabolites and their therapeutic aspects. In the first two chapters, South Korean and Indian authors have elaborated on the understanding about molecular mechanisms behind orientin- and plant flavonoids-mediated colorectal cancer prevention. Chapter 3 by Indian investigators discusses on plant neoflavonoids chemistry and biological functions. The role of polyphenols in autoimmune and chronic inflammatory diseases and the advent of computer-driven plant therapies are discussed in Chap. 4 by Pereira from the UK. In Chap. 5, the Mexican authors provide detailed information related to plant-derived alkaloids, their structural classification, and bioactive properties. While Indian authors in the next chapter provide detailed information about plant-derived bioactive molecules in treating breast cancer. Chapter 8 is a joint venture of authors from Saudi Arabia and India, and it has reviewed the recent literature reports regarding chromene as a privileged scaffold in medicinal chemistry. Indian authors in Chap. 9 provide a detailed knowledge about the chemistry and application of natural coumarins. Anticancerous properties and diverse molecular effects exhibited by the pomegranate peel and its phytoconstituents are detailed in Chap. 10. It is followed by Chap. 11, which discusses about the general chemistry of the compound, lycopene, the pathways involved in its synthesis, its role in benefiting the human health, and mechanisms of action. In Chap. 12, Indian authors have addressed the helpful properties of phytochemicals and their mechanisms of action in mitigating the cataract formation. It is followed by the chapter of Malaysian authors, where bioactive xanthones from the medicinal plant, *Garcinia mangostana,* are described. The next two chapters (Chaps. 14 and 15) have been focused on the sources and types of capsaicinoids, and the mode of action of capsaicin against cancer, cardiovascular diseases, obesity management, pain relief, and urological disorders. Similarly, Chap. 16 discusses on various phytochemicals involved in cancer cure, while Chap. 17 highlights the anticancer and chemopreventive effects of various phytochemicals isolated from cruciferous plants by Indian authors. In Chap. 18, the authors from Mauritius provide a mechanistic overview of some common essential oil constituents with regard to their pharmacological properties. An updated information on neuroprotective plant-derived compounds and their mode of action is provided in Chap. 19 by the researchers of Portugal. Chapter 20 emphasizes on the anticancer activity of *Oroxylum indicum* plant extract and its major compound, baicalein, and also highlights their mechanisms of action on different cancer cells. Bioactive components and bioactivities of commonly found Indian tropical fruits are discussed in detail in Chap. 21. Likewise, flavanoids derived from *Citrus* species and their role in preventing cardiovascular diseases are discussed in Chap. 22. In the next chapter, bioflavonoids as a promising antiosteoporotic agent is discussed. Later, in Chaps. 24 and 25, applications of advanced bioinformatics tools and methods for designing, optimization, and high throughput screening of phytocompounds are detailed. In Chap. 26, Malaysian authors have used *in silico* molecular docking studies on glycyrrhizin, a potential drug candidates for cancer treatment.

Overall, the chapters included in this volume clearly indicate that plants are important sources of bioactive compounds, and many of them are claimed to be effective against various diseases. Phytocompounds exhibit their pharmacological properties in a different manner via one or more mechanisms of action. The book chapters mainly include highly valued and most desired plant bioactive metabolites, largely addressing on their resources, chemistry, biological properties, and molecular mechanisms of action. I applaud the editor, Dr. Mallappa Kumara Swamy for his magnanimous academic determinations in bringing this book volume.

Shahjahanpur, Uttar Pradesh, India Mohd. Sayeed Akhtar

Preface

Plant-derived products are the most suitable products that are safe to be used as an alternative medicine. Much before the use of allopathic medicines, plant-based products were commonly used globally for improving health conditions. Based on their traditional uses and experimental evidences, various bioactive phytoconstituents have been isolated or extracted from the medicinally important plants. Medicinal and aromatic plants serve as an important source of bioactive compounds. Plant-derived compounds are proven to have significant pharmacological effects and are very useful in the treatment of various infectious diseases. Several well-known therapeutically much-admired compounds, including gymnemic acids, kaempferol, coumarins, taxol, podophyllotoxins, luteolin, rosmarinic acid, camptothecin, vinblastine, vincristine, ginsenosides, etc., are being isolated and purified from medicinal plants. Though several phytocompounds have been isolated and explored for their biological activities, many such compounds have to be proved scientifically. Yes, discovering new lead molecules from plant sources is very necessary in order to combat the increasing incidences of new diseases in recent times. Thus, exploring the biosynthesized chemical compounds from plants offers a great prospect towards the drug discovery and development.

Phytochemicals exhibit their therapeutic benefit via different mechanisms of action. For instance, they may act as antioxidants and/or nutrient protectors and inhibit the formation of cancer-causing agents in our body. They can function as anticancer agents by improving the immune system, reducing inflammation, preventing DNA damage, and facilitating DNA repair, thereby slowing down cancer cell growth, regulating hormones, and preventing damaged cells from reproducing. Plant polyphenol's antioxidant capacity is demonstrated via their ability to modulate signalling pathways responsible for removing free oxygen and nitrogen species, mitigating the enzymatic promotion of pro-inflammatory activity. Likewise, alkaloids also have the potential to be antidiabetic agents due to their modulation of blood glucose and lipid content. Thus, plant-derived bioactives function in different ways to offer numerous health benefits. However, the proper knowledge and exploration of these isolated molecules or products could provide an alternative source to reduce health risks. The enlightenment of molecular mechanism provides an overview to understand the basic causes and consequences involved in the disease diagnosis and their prevention using targeted therapy. Exploring the basic cellular and molecular processes involved in the progression of the disease could be used as the

target for their preventive therapies. Though scientific evidences have suggested many plant compounds with chemopreventive potential, understanding the issues related to exposure time, bioavailability, toxic effects, and mechanisms of action will certainly help to identify the leads and utilize them against various diseases. There is an exceptional task to fulfill the growing demand for phytoconstituents by flavor and fragrance, food, and pharmaceutical industries.

In this book, a description on the basis of bioactive plant compounds, their chemical properties, and pharmacological effects against various human diseases and applications in drug, cosmetic, and herbal industries is given. Besides this, the book chapters highlight on the mechanisms of action of few phytocompounds with therapeutic potential. This book provides a good information to students, teachers, and healthcare professionals, who are involved in natural product research, phytochemistry, and pharmacological investigations. I am grateful to all the contributors of this volume for sharing their knowledge and research. I thank Dr. Mohd. Sayeed Akhtar for his recommendations to improvize the book volume and for writing the foreword for this volume. Also, I appreciate the group of Springer Nature for their support at every stage of the book publication.

Bengaluru, Karnataka, India Mallappa Kumara Swamy

About the Book

Plants produce a plethora of bioactive compounds with different chemical scaffolds. Plant-derived bioactive compounds modulate a diverse range of molecular targets and are used as drugs for treating numerous diseases. Most of the present-day medicines are derived either from plant compounds or their derivatives. Even today, plant compounds continue as a limitless reserve for the discovery of new medicines. Different classes of plant compounds like phenolics, flavonoids, saponins, alkaloids, etc. and their potential pharmacological applications are being explored, and many such compounds potential curative properties are yet to be understood in detail.

This book, *Plant-derived Bioactives,* is a collection of detailed information, involving research findings related to plant-derived bioactive compounds, and is brought in 2 volumes. Volume 1, entitled *Plant-derived Bioactives: Chemistry and Mode of Action* includes several interesting topics related to the chemistry of highly valued plant bioactive compounds, and their mode of actions at the molecular level. Volume 2, entitled *Plant-derived Bioactives: Production, Properties, and Therapeutic Applications* includes topics related to the sources, biosynthesis, production, biological properties, and therapeutic applications of plant bioactives.

Overall, the collection of information in these books will be advantageous to the scientific community to further explore different medicinal plants and their bioactive compounds for therapeutic applications. These books will be useful for scholars, teachers, and scientists involved in plant product research and shall facilitate them to innovate novel drugs.

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About the Editor

Mallappa Kumara Swamy is working as a Professor and Head, Department of Biotechnology at East West First Grade College (Affiliated to Bangalore University), Bengaluru, India. He has completed his Postdoctoral Research at the Department of Crop Science and was Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Malaysia, from 2014 to 2018. Before that, he had worked as an Associate Professor and Head, Department of Biotechnology at Padmashree Institute of Management and Sciences, Bangalore University, Bengaluru, India. He obtained his PhD (Biotechnology) from Acharya Nagarjuna University, Guntur, India, in 2013. He is having more than 16 years of teaching and research experience in the fields of plant biotechnology, secondary metabolites production, phytochemistry, and bioactive studies. To his credit, he has published more than 100 research publications in peer-reviewed journals and 25 book chapters with reputed book publishers. So far, he has edited 10 books with Springer Nature Singapore Pte Ltd., CRC Press (Taylor & Francis Group), USA, and Studium Press, India. He is also serving as the Editorial board member and reviewer for few high impact international journals. Presently, he is working on the area of natural products research, plant cell and tissue culture technology for bioactive compounds production and evaluation of their bioactivities. Also, his research is focused on nanobiotechnology for medical applications.

Orientin: A C-Glycosyl Flavonoid that Mitigates Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is one of the most frequent malignancies, causing human deaths in large numbers around the world. It can be prevented by using chemotherapeutic drugs; however, the available drugs have shown systemic toxicity and drug resistance, which makes the treatment a challenging issue. The use of flavonoid-rich sources may effectively reduce the risk of colorectal cancer. Orientin, a C-glycosyl flavonoid exists in diverse medicinal flora, such as *Aspalathus linearis*, *Ocimum tenuiflorum*, *Passiflora*, and *Phyllostachys* species, and it has been shown to have beneficiary effects in treating cancers, cardiovascular diseases, and neurodegenerative disorders. Recent preclinical validation studies on orientin have evidently shown its anti-carcinogenic effect against human colorectal adenocarcinoma cells and carcinogen-induced CRC albino Wistar rat models. Orientin reinstates the antioxidants to put forth their scavenging mechanism and limits the activation of phase 1 enzymes. Orientin induces mitochondrial intrinsic apoptosis in CRC cells, and thereby actively interrupt cell proliferation and inflammatory signaling pathways without disturbing the normal tissue. Research reports have reported the attenuating effects of orientin on aberrant crypt foci progression in cancer-bearing animal, and it results in a significant suppression of pre-neoplasia to malignant neoplasia transformation. Orientin also suppresses NF-κB and the associated inflammatory cytokines, and thereby ameliorates inducible nitric oxide synthase and cyclooxygenase-2 expression in 1,2-dimethylhydrazine rat models. Thus, this chapter emphasizes the therapeutic effects of flavonoids with a special focus on orientin against CRC. Also, it summarizes our understandings about the molecular mechanisms behind orientin-mediated cancer prevention.

Keywords

Colorectal cancer · Orientin · Chemoprevention · Apoptosis · Cell proliferation

1.1 Introduction

Chemoprevention involves the inhibition or delay in the development of neoplasia by interrupting the instigation of neoplasia as well as transformation before malignancy. Colorectal cancer is probably a preventable cancer. The chemoprevention of colorectal cancer (CRC) has been the spotlight of research for three decades, which intends to thwart or delay the commencement of cancer through the deterioration or anticipation of colonic adenomas (Ricciardiello et al. 2016). Chemotherapy is highly recommended for colorectal cancer patients as palliative and adjuvant chemotherapy. It also depends on the tumor staging of CRC. Adjuvant chemotherapy is optional for patients under the stage III and in some cases for stage II patients with a chance of recurrence after the surgery. Palliative chemotherapy is optional for the stage IV patients, when the cancerous cells spread from the colon to other organs and tissues that are even far apart. 5-Fluorouracil, irinotecan, capecitabine, oxaliplatin, tipiracil, and trifluridine are some of the common chemotherapeutic agents that are used against CRC (Idrees and Tejani 2019; Akhtar and Swamy 2018).

A huge number of chemopreventive agents demonstrated promising results in preclinical models; however clinical trials have succumbed to contradictory outcomes. The detrimental effects of some of the clinically proven antitumor compounds boost up death cases and accentuate a vital need for novel and nontoxic chemopreventive agents (Youns and Hegazy 2017; Bhatia and Nair 2018; Roy et al. 2018). Many known natural compounds are notorious to exert significant inhibitory action against aberrant signaling pathways involved in carcinogenesis. Furthermore, the natural compounds can be obtainable readily. They are cost-effective and harmless with both protective and health-giving potential against cancers (Arivalagan et al. 2015; Bhatia and Nair 2018). The compounds of natural origin, such as dietary agents, have been of immense interest due to the development of novel and innovative therapeutic agents for cancer (Karthi et al. 2016; Tuorkey 2016; Roy et al. 2018). In this regard, medicinal plants have an immense potential of providing natural agents, i.e., secondary metabolites that are effective in treating several types of cancers. Among plant-derived metabolites, flavonoids are found to have several medicinal importance (Chang et al. 2018; Anwar et al. 2019; Jain et al. 2019). Flavonoids like umbelliferone, luteolin, orientin, and eriodictyol have been shown to have considerable antiproliferative activities against cancerous cells. Reports have shown the attenuating effects of orientin on aberrant crypt foci (ACF) progression in cancer-bearing animal, and it results in a significant suppression of preneoplasia to malignant neoplasia transformation (Muthu et al. 2016; Mariyappan et al. 2017; Thangaraj et al. 2018). Orientin has also been shown to suppress NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and the associated inflammatory cytokines and thereby ameliorates inducible nitric oxide synthase and cyclooxygenase-2 expression in 1,2-dimethylhydrazine rat models (Hamiza et al. 2012a, b; Thangaraj et al. 2018). Thus, this chapter emphasizes the therapeutic effects of flavonoids with a special focus on orientin against CRC. Also, it summarizes our understandings about the molecular mechanisms behind orientin-mediated cancer prevention.

1.2 Flavonoid's Role in Cancer Prevention

Flavonoids possess the potential of regulating multiple carcinogenic processes, such as apoptosis, angiogenesis, tumor differentiation, and cell proliferation. The flavonoid-induced alterations in kinase activity have a strong relationship with apoptosis, tumor cell proliferation, and invasion. Some of the dietary flavonoids have displayed in vivo antitumor activity and repress inflammation, proliferation, vascularization, and metastasis (Kumar and Pandey 2013; Panche et al. 2016; Tungmunnithum et al. 2018). Epidemiological studies on colon cancer proposed that some flavonoids that prevent colon cancer may enhance therapeutic efficiency by regulating colon tumor cell proliferation and survival signaling pathways (Zamora-Ros et al. 2015; Thangaraj et al. 2018). Flavonoids block several steps in carcinogenesis, namely tumor cell transformation, invasion, and metastasis (Chahar et al. 2011). A positive correlation exists between a flavonoid-rich diet and lowers the risks of colon cancer. Several flavonoids, such as umbelliferone, luteolin, and eriodictyol have been shown to exhibit superior inhibitory activities against colorectal cancer cells (Manju and Nalini 2010; Muthu et al. 2016; Mariyappan et al. 2017). Naturally available flavonoids most commonly exist as O- or C-glycosides. C-Glycosylflavones are the major class of flavonoids, which imparts the dark-yellow color to flowers of Leguminosae family members. C-Glycosides are well known for their diverse pharmacological potentials, including antioxidant, antiinflammatory, antimicrobial, and antitumor activities.

1.3 About Orientin

1.3.1 Chemistry

The IUPAC name of orientin is 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one. Orientin is a water-soluble C-glycosyl flavonoid with the molecular formula $C_{21}H_{20}O_{11}$, and its molecular weight is 448.38 g/mol. In general, C-glycosyl flavonoids possess C_6–C_3–C_6 flavone skeleton, in which two aromatic rings are attached by three carbons cyclized with oxygen, having one to several glucoside units. Orientin has a 15-carbon skeleton, consisting of two phenyl rings (A and B); in specific O-phenolic hydroxyl group at 3′ of B ring and a heterocyclic ring exemption of a C-glycosides linked to glucose of the A ring (An et al. 2015). It comprises phenol groups alongside one ketone group and two ether groups. It is the 8-C glucoside of luteolin, substituted by a β-D-glucopyranosyl moiety at position 8.

1.3.2 Natural Occurrence

Several medicinal plants are the source of orientin. Some of the most widely explored medicinal plants for orientin include *Ocimum sanctum* (holy basil) (Devi

et al. 2004), *Aspalathus linearis* (rooibos tea) (Koeppen et al. 1962), *Phyllostachys nigra* (bamboo leaves), *Passiflora edulis* (passion flowers, juice and peel of passion fruit), *Trollius chinensis* Bunge (Golden Queen), *Linum usitatissimum* (flax) (Dubois and Mabry 1971), *Jatropha gossypifolia* (bellyache bush), *Commelina communis* (dayflower) (Shibano et al. 2008), *Euterpe oleracea* Mart. (acai palm) (Gallori et al. 2004), *Ascarina lucida* (Soltis and Bohm 1982), *Roscoea capitata*, *Celtis africana* (white stinkwood) (Perveen et al. 2011), *Croton zambesicus* (lavender croton) leaves (Wagner et al. 1970), *Cajanus cajan* (pigeon pea) leaves (Pal et al. 2011), *Thlaspi arvense* (field penny-cress) (Pang et al. 2013), *Fagopyrum esculentum* (buckwheat) (Krahl et al. 2008), *Trigonella foenum-graecum* L. (fenugreek) oils (He et al. 2014), *Clinacanthus nutans* (Sabah snake grass) leaves (Chelyn et al. 2014), and *Polygonum orientale* L. (Li et al. 2017a).

1.3.3 Pharmacological Properties

Orientin has been testified to possess numerous medicinal properties, such as anti-aging, antiviral, antibacterial, antiinflammation, cardioprotective, antinociceptive, radioprotective, and neuroprotective effects. Orientin inhibits esophageal cancer (EC-109) cell development in a time-dependent and dose-dependent way. It has been found to downregulate the anti-apoptotic B-cell lymphoma 2 (Bcl-2) expression and induce early apoptosis in EC-109 cells. Orientin was found to have higher antitumorigenic effect, when compared to vitexin (an apigenin flavone glycoside). The researchers have doubted on the associated OH-groups at the 3′- and 4′-position of the B ring in orientin for the increased effect. Due to the presence of a single OH-group at the 4′-position of the B ring in vitexin, its was believed to be less effective (An et al. 2015). Orientin inhibited the multiplication of HeLa (cervical cancer) cells in a concentration-dependent way and stimulated apoptosis. It reduced anti-apoptotic Bcl-2 and enhanced pro-apoptotic Bax (bcl-2-like protein 4) protein levels in HeLa cells. Also, it instigated the proteolytic stimulation of protease enzymes, i.e., caspases (Guo et al. 2014). Orientin induces apoptosis and inhibited the proliferation of breast cancer (MCF-7) cells in a time- and dose-dependent way (Czemplik et al. 2016). However, so far, none of the information validates the influence of orientin against CRC. Further, the precise molecular mechanisms of action of cell inhibitory activities induced by orientin still remain unclear.

1.4 Experimental Colorectal Carcinogenesis

1.4.1 HT29 (Human Colorectal Adenocarcinoma) Cell Line

HT29 is a human colon adenocarcinoma with epithelial morphology. This cell line was firstly obtained from a Caucasian woman (aged 44 years) suffering from a cancer of the colon (Fogh and Trempe 1975). HT29 cells contain unique features, such as microvilli, microfilaments, lipid droplets, smooth and rough endoplasmic

reticulum with free ribosomes, large vacuolated mitochondria with dark granules, and few primary and many secondary lysosomes (Martínez-Maqueda et al. 2015). These cells consume high levels of glucose, and hence in vitro growth of these cells needs a medium containing elevated levels of glucose. They propagate as a multilayer of nonpolarized cells under standard conditions. After the treatment with inducers, these cells can be modulated to express various paths of absorptive cell differentiation, such as cell flattening, apical surfaces with brush border development, and formation of tight junctions among the adjoining cells. These intestinal cells are pluripotent and widely used to study cell differentiation mechanisms (Martínez-Maqueda et al. 2015). The p53 (a cellular tumor protein) antigen is overproduced in these cells. HT29 secretes pro-inflammatory cytokines, namely, tumor necrosis factor-α (TNF α) and interleukins (IL-1β and IL-6); chemokines (e.g., interferon-γ and IL-8); transforming growth factors (TGF-α and TGF-β); pro-angiogenic factors like vascular endothelial growth factor (VEGF) and IL-15; and immune-modulatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (GCSF), and IL-3 (Desai et al. 2013).

Orientin expressively inhibits the cell survival of HT29 in a concentration-dependent way. Further, microscopic interpretations suggested the cell shrinking from its polyhedral origin, membrane blebbing, round off, and cells in detached forms (Karthi et al. 2016). The oxidative stress-induced intracellular ROS (reactive oxygen species) generation in tumor cells serves to be one of the possible therapeutic strategies for combating CRC. Excessive generation of ROS may severely damage the genome and proteins, resulting to promote apoptosis (Han et al. 2013). Orientin triggers dose-dependent intracellular ROS generation extensively.

1.4.2 Chemically Induced Animal Models

Chemical carcinogens are extensively used to induce colonic epithelial lesions similar to human CRC. They include aromatic amines, 1,2-dimethylhydrazine (DMH), heterocyclic amines, azoxymethane (AOM), and alkylnitrosamide compounds. These carcinogens can be readily verified for their therapeutic effects in model animals of diverse genetic conditions, and the human CRC pathogenesis can be initiated successfully (De Robertis et al. 2011). The anticancer efficacy of chemopreventive agents is generally tested against carcinogen-induced preclinical models. The rodents (rat/mouse) are commonly established animal models of colon carcinogenesis. This is because their physiology is similar to humans. Also the cancer formation is rapid and reproducible and there is a possibility of studying adenoma-carcinoma sequences (Muthu et al. 2016).

1.4.2.1 DMH-Induced Experimental Colon Carcinogenesis

The procarcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) was extensively used to stimulate colon tumors in rodents. It mimics human colon carcinoma in epithelial origin, morphology, anatomy, histology and tumor characteristics, thereby

acting as an ultimate experimental model for chemoprevention studies (Manju and Nalini 2005; Nirmala and Ramanathan 2011; Muthu and Vaiyapuri 2013). DMH induced the transformation of pre-neoplastic aberrant crypt foci into adenomas and adenocarcinomas. DMH and its inter-metabolites (AOM and MAM) are a set of man-made composites with the general structure of cycasin (Liu et al. 2015). DMH uptake is threefold higher in the colorectal cells in contrast to the enterocytes. The cancer-causing effect of DMH occurs with a single dosage of injection or by giving a series of injections after every week. DMH injected at a dose between 10 and 20 mg/kg body weight (BW) produces adenomas and adenocarcinomas of the colon in rodents. After the administration, the malignant lesion is formed from the non-dysplastic mucosa, and later it completely develops within 4 to 30 weeks. About 80% of the treated mice can develop adenocarcinomas even when a lesser dose of drugs are administered (Machado et al. 2016). Injecting a small dosage (10 mg/kg) of dimethylhydrazine will lead to colorectal cancer development in rats with a latency duration of 1–2 years (Banerjee and Quirke 1998). The preneoplastic lesions namely the aberrant crypt foci (ACF) are the earliest marker of future neoplastic development that appears after exposing DMH for 14 days (Kilari et al. 2016).

1.4.2.2 Metabolism of DMH

DMH undergoes dehydrogenation in the liver and is metabolically activated to azoxymethane (AOM) and methylazoxymethanol (MAM) (Manju and Nalini 2010). Generally, DMH do not act as carcinogenic agent. However, it is metabolically activated (including N-oxidation and hydroxylation) into DNA reactive metabolites and exhibits its tumorigenic potential (Qi et al. 2015). The highly unstable MAM with a half-life of 12 h is metabolized further to form an active electrophilic methyldiazonium glucuronide by NAD + -dependent dehydrogenase. The methyldiazonium ion which has the capability to alkylate macromolecules excretes via bile and blood to reach the colonic lumen.

Cytochrome P450 enzymes are involved in the bioactivation of procarcinogens as they interfere with the polar functional groups via hydrolysis and redox mechanisms. The phase 2 enzymes, such as glutathione S-transferase (GST), N-acetyltransferase (NAT), DT-diaphorase (DTD), UDP-glucuronosyltransferase (UGT), and sulfotransferase (SULT), detoxify upon conjugation (Beyerle et al. 2015). Cytochrome P450-dependent monooxygenases (phase 1 enzymes) carry out a reaction to introduce –OH groups to produce methyldiazonium ions, which are highly reactive in nature, and this in turn alkylates DNA bases. The strong nucleophilic methyldiazonium ions interact with nucleotide bases to yield adducts. Both N^7-methylguanine and O^6-methylguanine induce the genetic mutations and tumor formation (Megaraj et al. 2014). O^6-Methylguanine induces GC to AT transitions and k-ras protooncogene mutations in DMH-induced colorectal carcinogenesis. Phase 2 enzymes, such as GST and DTD, are known to detoxify the electrophilic intermediate compounds (Giftson et al. 2010).

1.5 Orientin Against Colon Cancer in Different Ways

1.5.1 Body Weight, Growth Rate, and Polyp's Incidence

Body weight and growth rate typically controls the carcinogenicity rate in carcinogen-induced investigational animals (Manju and Nalini 2005). Loss in body weight in addition to growth rate in DMH alone induced rats could be because of increased cancer burden, lack of appetite followed by the higher incidence of polyps driven cachexia and anorexia (Vinothkumar et al. 2014a). Orientin increases the body weight despite the transformation induced by DMH, owing to their capability to reinstate the metabolic deregulation. The plant metabolites have been proven to gain body weight by restraining the cancer-causing agent-induced tumorigenicity and diminishing the incidence of polyps (Selvam et al. 2009).

1.5.2 Lipid Peroxidation and Antioxidant Status

Antioxidant and lipid peroxidation levels are anticipated to be the notorious markers for ascertaining the peril of oxidative damage-induced tumorigenesis (Thangaraj et al. 2018; Muthu and Vaiyapuri 2013). The significant increase in circulatory thiobarbituric acid reaction substances (TBARS) by the high ROS production and membrane crumbling leads to the transformation of epithelial cells (Perše 2013). Tumors acquire favorable conditions and proliferate rapidly in DMH-exposed rats where the lipid peroxidation is decreased (Giftson et al. 2010; Ghadi et al. 2009; Vinothkumar et al. 2014b). The decreased TBARS in colon tissues might be because of the concomitant resistance and reduced vulnerability of tumor cells to the ROS scavenging action (Muthu and Vaiyapuri 2013). Antioxidants defend cells from the oxidative damages via free radical scavenging activities. The antioxidants, such as CAT and SOD, primarily scavenge the reactive oxygen species; GPx detoxifies H_2O_2 and by this means counteracts reactive oxygen species. GSH and the dependent enzymes closely associate with innate defense (Siddique et al. 2017). The increased consumption of tissue antioxidants during DMH metabolite detoxification in tumor cells leads to their decreased level in DMH-alone-exposed rats. Orientin restores the antioxidant levels to put forth their scavenging action. The free -OH groups present in orientin make it an efficient antioxidative agent against ROS prompted by DMH and thereby exhibit their inhibitory prospective against colorectal carcinogenesis.

1.5.3 Xenobiotic Metabolizing Enzymes

DMH upon dehydrogenation in the liver forms azoxymethane (AOM) and methylazoxymethanol (MAM) intermediates (Manju and Nalini 2010). Phase 1 and phase 2 enzymes introduce polar or reactive groups into xenobiotics or carcinogens. Phase 1 enzymes stimulate the procarcinogens by introducing polar active groups, while the phase 2 enzymes detoxify after conjugating with the carcinogens (Padmavathi

et al. 2006; Beyerle et al. 2015). Cytochrome P450 enzymes effectively convert lipophilic xenobiotics into more hydrophilic carcinogenic compounds (Sangeetha et al. 2012). The increased level of liver microsomal drug-metabolizing enzyme and intestinal epithelial cell phase 1 enzymes, i.e., CYP2E1, CYP450, and cytochrome b, exhibit DMH-induced carcinogenicity. The genotoxic intermediates produced by phase 1 enzymes covalently attach to form DNA adducts (Balaji et al. 2014). Similar to many flavonoids, orientin administration activates cytochrome P450 enzymes owing to the existence of –OH groups (Sangeetha et al. 2012). The phase 2 enzymes, comprising GST and DT-diaphorase, aid in the introduction of a polar or reactive group to xenobiotic compounds. DT-diaphorase, a widely distributed flavoprotein in animal tissues, detoxifies the quinone and its derivatives to protect against neoplasia (Mohan et al. 2006). Glutathione-S-transferase (GST) interrupts initiation of carcinogenesis by detoxifying hydroquinones and neutralizing electrophilic intermediates (Balaji et al. 2014). The increased level of phase 2 enzymes aids protection against carcinogens. The decreased phase 2 enzyme levels in the hepatic and colonic regions might be because of utilizing more detoxifying enzymes for counteracting DMH-induced malignant tumor formation. Orientin increased the level of phase 2 enzymes and detoxifies the carcinogens. Orientin diminishes active DMH metabolite formation and excretes carcinogen from the colonic lumen.

1.5.4 ACF Formation

Aberrant crypt foci are surrogate precursor lesions of colon cancer distinguished from the usual crypts based on their size, shape, thickness, and the pattern of staining (Bird and Good 2000; Rodrigues et al. 2002). The aberrant crypt with high multiplication rate, i.e., >4 crypts/focus, and their number are the indication of colorectal cancer incidence (Aranganathan and Nalini 2009). The carcinogen-induced rats show higher incidences of ACF and cancer frequency (Baskar et al. 2011). The bioactive compounds inhibiting ACF would also promote the antitumorigenicity (Sengottuvelan et al. 2006; Muthu and Vaiyapuri 2013). ACF shows the initiation of colorectal cancer formation and their increased numbers and multiplicity of crypt suggest the advancement and progress of cancer. Orientin inhibits the ACF progression and suppresses the transformation to malignant neoplasia. The protective activity is ascribed to the antioxidative efficiency of orientin and the metabolic activation of xenobiotic enzymes. The capability of orientin to reinstate DMH-stimulated histological changes authenticates its anticancer and antiinflammatory potentials.

1.5.5 Tumor Marker Levels

An early identification and lessening of precancerous lesions can be made possible by the quantification of serum markers, which may curtail the incidences and death rate of CRC. The assessment of tumor marker levels in serum acts as the prominent

markers owing to their ease of handling and economical. The Serological cancer markers (CEA and CA 19–9) are synthesized and liberated into the interstitial fluid and then enters lymph to enter circulation (Narimatsu et al. 2010). Cells holding a higher metastatic potential express these intracellular adhesive proteins on their surface. CEA, the glycosylated immunoglobulin, is the most often characterized tumor-associated antigen and widely existing biomarker for CRC patients. CEA is found in high levels during the malignant and metastatic stages compared to benign conditions (Shitrit et al. 2005). CEA levels in the columnar epithelial cells and goblet cells vary with tumor staging (Flamen et al. 2001). The elevated levels of CEA found in DMH-stimulated experimental animals are most probably linked with tumor size, stage, multiplication, and tumor site. Orientin is reported to moderate CEA levels via reducing the rate of tumor development. CA 19–9 is one of more familiar cancer markers for diagnosing CRC. The increased level of this glycolipid is highly correlated with increased CRC mortality cases. The increased CA 19–9 levels is highly related to perineural and lymphatic invasions, leading to metastatic tumors (Fernandez-Fernandez et al. 1995). The decline in the levels of CA 19–9 after injecting orientin hypothesizes the antitumor action, similar to that of umbelliferone (Muthu and Vaiyapuri 2013). Moreover, orientin treatment for the whole period revealed a strong influence of its cancer preventive potential in DMH-prompted colorectal cancer-possessing experimental animals.

1.5.6 Mast Cell Infiltration

Infiltration of mast cells acts as the finest marker for beginning of inflammatory process. The innate cells of the immune system alter the progression of adenomas into carcinoma initiation by eliciting inflammatory reactions (Khan et al. 2013). Mast cell infiltration is apparently observed in the submucosal layer of DMH-treated animals, which aggravates constant inflammation in the tumor microenvironment. Orientin reduced mast cell infiltration to indicate that it has a potential antiinflammatory activity and also anti-angiogenic potential. The reported antiinflammatory effect of orientin was similar to carvacrol and umbelliferone (Muthu and Vaiyapuri 2013; Arivalagan et al. 2015).

1.5.7 Tumor Cell Proliferation

The reliability of intestinal mucosa is maintained by the crucial mechanism of cell proliferation. The deregulation of cancer cell multiplication recurrently effects in hypergenesis and oncogenesis (Lee and Yun 2010). The nucleolus-associated chromosomal regions (nucleolar organizer regions) are positioned on the acrocentric chromosomes short arm. NORs containing the acidic proteins are silver-stained (AgNORs), and they serve as the investigative or predictive marker of cell multiplication/proliferation (Gundog et al. 2015). In cancerous cells, the cell proliferation is positively linked to AgNORs/nucleus (Sengottuvelan et al. 2006). The total number

of AgNORs/nucleus determines cancer succession and the developmental phases. The black dots visualized are utilized as prognostic indicators for cell spread/propagation (Arivalagan et al. 2015). The aggregated numbers of AgNORs/nucleus in the colonic epithelium were found to be higher in DMH-treated experimental animals (Sengottuvelan et al. 2006; Muthu et al. 2016). Orientin decreases the number of AgNORs/nucleus owing to its role in preventing the proliferation of cancer cells. Umbelliferone was earlier reported to decrease the number of AgNORs/nucleus in enterocytes (Muthu et al. 2016; Mariyappan et al. 2017).

Proliferating cell nuclear antigen (PCNA), a non-histone nuclear acidic protein (of 36 KDa), is presumed as an intermediate biomarker of cell proliferation (Aranganathan and Nalini 2013). The expression of PCNA was high in the nuclei of multiplying cells in the growth (G1) phase and early synthetic (S) phase of cell cycle. PCNA monomers enclose the DNA strand like a ring and hold DNA polymerase δ (DNA Pol δ) to the template strand as a clamp to cause hyperproliferation (Mohania et al. 2014). Another proliferating antigen, Ki67 expresses only during G1, S, and G2 phases, but not in the G0 phase. The augmented nuclear expression of PCNA and Ki67 in DMH-induced rats indicates a high proliferation of colonic epithelial cells (Sekar et al. 2016). The suppression of cell proliferation represents one of the protecting actions against DMH-treated colon cancer. Orientin was shown to decrease the expressions of PCNA, Ki67, and their labeling indices. Orientin inhibits cell proliferation by its anti-apoptotic activities (Karthikkumar et al. 2015).

1.5.8 NF-κB and Inflammatory Cytokine Expression

Tumors develop and promote the inflammatory signals in and around the microenvironment. NF-κB activation allows translocation of NF-κB dimer to initiate the transcription of pro-inflammatory cytokines and the downstream target genes (Umesalma and Sudhandiran 2010). The degree of inflammations in DMH-treated animals was apparent by increased NF-κB expression. Orientin reduced the expression of NF-κB owing to its antiinflammatory potentials against DMH-treated inflammations. The inflammatory cytokines (IL-6 and TNF-α) are produced in the tumor microenvironment, which regulates proliferation and apoptosis. The increased TNF-α level during chronic inflammation upholds tumor propagation and metastatic behavior (Colussi et al. 2013). IL-6 is mostly formed by macrophages and monocytes, following the activation of NF-κB. IL-6 plays a huge role during the acute inflammation, tumor cell multiplication, and apoptotic mediated cell death (Dai et al. 2014). TNF-α and IL-6 inhibition serve one of the effective approaches in the treatment of CRC. The elevated production of cytokines during DMH-induced inflammatory response gets downregulated because of the inhibitory potential of orientin (Nash and Ward 2014).

1.5.9 Pro-Inflammatory Enzymes

Inducible nitric oxide synthase (iNOS), the pro-inflammatory mediator, synthesizes nitric oxide (NO), which arbitrates inflammation and persuades tumorigenesis. The carcinogen-induced tumor-bearing rats increased iNOS expression, thereby promoting the invasiveness and metastatic potential (Muthu et al. 2016). iNOS overexpression in colonic cells associates with CRC pathogenesis, where it restrains apoptosis via nitrosylation of caspases. NO impedes DNA repair mechanisms and leads to cytokine post-translational modifications which further influence the commencement and progression of CRC (Narayanan et al. 2003). Orientin distinctly improves the expression of iNOS in DMH-induced rats.

Cyclooxygenase enzymes (COX-2) catalyze prostaglandin synthesis from arachidonic acid, which gets induced at the inflammatory phase and overexpressed in colonic adenocarcinoma (Hamiza et al. 2012a, b). The inflammatory chemokines, cytokines, and tumor promoters induce COX-2 activation (Peng et al. 2013). COX-2 enzymes express higher in cancerous cells via the activation of NF-κB mediated inflammatory cytokine pathway. The increase in COX-2 expression in DMH induced rats was due to its anti-apoptotic effect on colon cancerous cells (Srimuangwong et al. 2012). Orientin distinctly decreased COX-2 expression and corroborates their inhibitory action against inflammation-linked colon tumorigenesis. Orientin suppress the overexpression of inflammatory cytokines due to DMH treatment, thereby validating their antiinflammatory and antiproliferative effects.

1.5.10 Cell Cycle Arrest

The cell cycle dysregulation and avoidance of apoptotic mediated cell death are the familiar events in CRC development (Wu et al. 2018). Arresting of cell cycle at a particular checkpoint and apoptotic induction are the most commonly used mechanisms in the chemoprotection of tumors (Song et al. 2017). The cell cycle checkpoints reinforce the differentiating cells from DNA damages and control the genomic integrity. Orientin stimulated cell cycle arrest at G0/G1 phase in a concentration-dependent way. The stimulated cyclin-dependent serine/threonine kinases and their regulatory cyclin subunits regulate cell cycle progression. These cyclin/CDK complexes act as a biomarker for tumor cell multiplication and targets for anticancer drug development (Peyressatre et al. 2015). Among the different cyclin/CDK complexes, cyclin D and cyclin E along with CDK2 and CDK4 control mitotic division and advance the cell cycle through G1 phase. Orientin noticeably reduced the expression of cyclin D1, cyclin E, and the cyclin-dependent kinases, CDK2 and CDK4. Pelargonidin also showed the reduced expression of CDKs and cyclins in colorectal cancer cell line, HT29 (Karthi et al. 2016). Orientin elevates $p21^{WAF1/CIP1}$, the chief inhibitor of cyclin D/CDK complex. $p21^{WAF1/CIP1}$ mediates G0/G1 phase arrest in HT29 cells (Kan et al. 2013). CDK4 activated phosphorylation of Rb trigger the disruption of tumor suppressors which further discharges E2F and

initiates G1 to S phase transition (Asghar et al. 2015). Orientin reduces the expression of pRb and thereby inhibits G1 to S phase transition.

1.5.11 Apoptosis

Apoptosis is a complex sequence, which regulates cell proliferation and protects cells from being malignant by getting rid of immortal or repaired cells. Multiple signals trigger the loss of mitochondrial membrane potential with the successive release of cytochrome C from cytosol in carcinogen-induced cancer cells (Lemieszek et al. 2016). Apoptosis is conceivably the powerful defense mechanism of many chemotherapeutic agents toward CRC. The evolutionarily conserved members of Bcl-2 protein family such as Bcl-2 and Bax regulate apoptosis. The pro-apoptotic Bax confines to mitochondria and triggered cytochrome C release leading to caspase-mediated cell death. The prosurvival Bcl-2 binds along with Bax to avert its oligomerization and thwarts mitochondrial membrane depolarization and thereby cytochrome C release (Ding et al. 2010; Tanwar et al. 2010). Orientin decreased the level of anti apoptotic Bcl-2 and Bcl-XL with the increased pro apoptotic Bax and Bid levels which obviously demonstrated the immense potential of Orientin in regulation of Bcl-2 family proteins and apoptotic induction in colon tumor cells. Orientin activates mitochondria-mediated apoptosis in DMH-induced CRC-bearing rats by the simultaneous increase of cytosolic cytochrome C with a decrease in Bcl-2/Bax ratio. Orientin increases caspase 3 and caspase 9 expression in tumor-bearing rats. Caspases are the aspartate-specific cysteine proteases that play a decisive role in apoptotic process. The binding of cytosolic cytochrome C with Apaf-1 activates caspase 9, the initiator caspase, and in turn activates the caspase 3, the downstream effector which leads to intrinsic apoptosis (Sengottuvelan et al. 2009). Orientin activates mitochondria-mediated intrinsic apoptosis in DMH-induced CRC-bearing rats.

Orientin induces Smac/DIABLO release in HT29 cells, along with cytochrome C, thereby neutralizing inhibitor of apoptosis proteins (IAPs) (Srinivasula et al. 2000; Endo et al. 2009; Abdel-Magid 2017). The cytochrome C associates with apoptotic protease-activating factors (Apaf-1) and procaspase 9 to form apoptosome and commence the activation of caspase cascade. The binding of Apaf-1 triggers a conformational change in procaspase 9 to caspase 9 (Omer et al. 2017). Orientin increases caspases (caspase 9 and caspase 3) and cleavage of poly(ADP-ribose) polymerases. Orientin induces apoptosis primarily in the intrinsic pathway (Jiang et al. 2017; Li et al. 2017b). Orientin decreased the expression of inhibitor of apoptosis protein family members, XIAP and Survivin, in HT29 cells due to the release of cytochrome C and the depolarization of mitochondrial membrane potential (Abdel-Magid 2017). Orientin increased expression of tumor suppressor p53 and induced overexpression of $p21^{WAF1/CIP1}$. The increased level of γ-H2AX in Orientin treated tumor cells serves as a hallmark of DNA damages which confirms the DNA damage induced in HT29 cells.

1.6 Conclusions

Colorectal cancer is one of the most diagnosed cancers, which can be protected or prevented with the chemotherapeutic agents; however, the available drugs have shown systemic toxicity and drug resistance which makes the treatment a challenging issue. A positive correlation exists between flavonoid-rich sources and lowers the risk of colorectal cancer. The above discussed contents explored the effect of dietary flavonoid, orientin, and validated its anti-carcinogenic effect in human colorectal adenocarcinoma HT29 cells and DMH-induced colorectal cancer-bearing Wistar rats. Orientin induces mitochondria-mediated intrinsic apoptosis in colorectal cancer cells and arrests tumor growth by interrupting cell proliferation and inflammatory signaling pathways. Orientin exerts a regulatory effect on major signaling pathways linked with colon cancer progression, namely PTEN/PI3/Akt and Wnt/β-catenin pathways. Overall, this chapter suggests that orientin can be a novel chemotherapeutic agent for controlling CRC.

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Plant Flavonoids Against Colorectal Cancer and Mechanisms of Action

2

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Abstract

Worldwide colorectal cancer (CRC) was considered to be a primary health disease that leads to high morbidity and mortality in both developed and developing countries, which can be controlled with novel chemopreventive agents. In the past few decades, chemoprevention of several cancers by the use of natural flavonoids has become an attractive strategy. Bioactive polyphenols with a low molecular weight in the dietary supplements determine the fate of a cell by interacting at the molecular and cellular level. In the past few decades, several preclinical/clinical studies perceptibly figured the chemopreventive and cytotoxic effect of bioactive flavonoids on cancers. Flavonoids have been frequently considered as enzyme inhibitors and ligands of receptors involved in the signal transduction. There has been increasing interest in unrevealing the beneficial and chemoprotective potential of flavonoids that had encouraged in novel drug discovery for CRC. An elaborated molecular mechanism of phytocompounds against cancer treatment overlies with direct interactions between various types of genes and enzymes. It is well known that anticancer efficacy would include apoptosis, antiproliferation, antioxidation, cell cycle arrest, and reversal of multidrug resistance mechanism. This chapter highlights the cumulative research findings of plant-derived bioactive flavonoids against CRC treatment and provides novel insights into its molecular regulation and mechanism with protein interaction in anticancer drug discovery.

Keywords

Flavonoids · Anticancer · Colorectal cancer · Drug discovery · Bioactive compounds

2.1 Introduction

Bowel cancer has been diagnosed as the third most prevalent cancer worldwide. It is reported globally with a very high rate of prevalence and mortality in both sexes. Study literature pinpoints westernized life anticipation, unbalanced diet, uncooked food, processed and canned meats, change in environmental conditions due to urbanization, and synthetic mutagens due to pollution were thought to be a serious etiology of colorectal cancer (CRC). The key factors behind CRC etiology were denoted as oxidative stress caused by the generation of non-neutralizable reactive oxygen species (ROS) and free radicals, which can directly interact with the bio-molecules of living cells and induce spontaneous mutations in bowel cells that leads to colon cancer (Mariyappan et al. 2017). Subsequent accumulation of sequential genetic mutation in a colonic gene, in particular, oncogenes and tumor suppressor genes resulted in the stepwise progression of colon cancer that starts from bowl inflammation followed by the formation of adenomatous polyposis coli and finally leads to colorectal carcinogenesis (Fearon and Vogelstein 1990). Further, intensive

study reports on colorectal cancer in rodent models have stated that microflora of animal guts have a major contribution to the development of colon cancer (Manju and Nalini 2005). Environmental pollutants, chemical toxicants in food, and modernized dietary food style have a direct impact on the microenvironment of gut microbes. Such uncomfortable gut environment resulted in improper metabolism and development of resistance against deadly pathogens and procarcinogens along with unbalanced homeostasis of increased immune response that had extensively known to regulate T cell development and Th17 responses that were denoted as key factors for colon cancer development (Kamada et al. 2013). As per the previous reseach study, various epidemiological case studies had evidently proved that daily diet accounts a dominant role in etiology of CRC; in particular, processed meat and red meat consumption had evidently increased the risk of colon carcinogenesis, whereas consumption of fiber-rich food like fruits, vegetables, and whole grains had highly decreased the colon cancer risk (McGirr et al. 2017). As a highlight of this chapter, consumption of a variety of green leaves, vegetables, and fruits with high fiber contents also includes a variety of naturally occurring bioactive molecules scientifically termed as dietary flavonoids that possess a strong chemoprotective effect against colon cancer and also have inverse action on colon carcinogenesis mechanism (Chang et al. 2018). For decades, scientists have been working with the isolation and characterization of a number of naturally occurring flavonoids and elucidated its pharmacological properties on various study models. Therefore, this chapter particularly provides a brief review of a few such flavonoids along with its biological properties and protective mechanism of action against colorectal cancer.

2.2 Bacterial Enzymes and Colon Cancer

The human digestive tract inhabits approximately about 400 species of bacteria, and it is a complex ecosystem of multiple microorganisms. The literature review provides evidence that invasion of pathogenic bacteria into the intestine was prevented by the presence of anaerobic gut bacteria which in turn reduce the threatened occurrence of CRC, and it is because of bacterial conversion of short-chain fatty acids by dietary fiber (Abeni et al. 2013). Consumption of high-fat and imbalanced diet alters the protective metabolism of intestinal microflora by increased bacterial enzyme activity, which resulted in a decreased host immune responses (Asha and Gayathri 2012). One of the best examples is β-glucuronidase, a common gut bacterial enzyme that produces the toxic metabolites with carcinogenic effect, which has a significant role in the progression of the tumor at secondary sites like large intestine, colon, etc. (Beaud et al. 2005). 1,2-Dimethylhydrazine (DMH) is an alkylating agent which stimulates the formation of methyl adducts with DNA bases that leads to point mutation. It has been well studied that β-glucuronidase enzymes catalysis the conversion of procarcinogen form of DMH into various metabolic intermediates which has a higher efficacy to interact with colon epithelium and act as potent carcinogens (Reddy et al. 1974). The accumulation of aglycones is due to the catalytic activity of β-glucosidase which synergistically hydrolyzes cellulose to lignocellulose

(Sohail et al. 2009; Jeng et al. 2011). On the other hand, the permeability of the colon mucosal membrane was highly altered by the enzymatic catalysis of intestinal mucin by gut bacterial mucinase enzyme (Robertson et al. 1940). Along with other bacterial enzymes, nitroreductase also has a unique task in colon cancer development by producing amines in the colon tissues by catalytic conversion of procarcinogens such like nitropyrenes, dinitrotoluene, and nitrobenzenes (Facchini and Griffiths 1981).

2.3 Reactive Oxygen Species (ROS) and Cancer

Mitochondrial oxidative phosphorylation generates a large amount of ROS by impaired transfer of electrons between complexes I and III of the mitochondrial respiratory chain (MRC). ROS is a collective term that refers to highly reactive oxygen molecules that can be listed as hydroxyl radical (OH−) ions, hydrogen peroxide (H_2O_2), and superoxide anion (O_2-) (Fulda et al. 2010). Naturally occurring antioxidant enzymes such as superoxide dismutase enzymes and catalase serve as defense system by catalytically naturalizing the generated reactive pro-oxidants. ROS may have beneficial effects on regulation of cell proliferation and gene expression, and increased ROS production results in oxidative stress-induced damage to proteins, lipids, and DNA, which has implications in diseases including diabetes, Parkinson's disease, and cancer (Gogvadze et al. 2009). Studies of aging mechanism had revealed the role of oxidative stress in higher probability of risk for cancer development in old age. Generated reactive species or free radicals actively damage genomic or mitochondrial DNA that leads to sudden mutation implicated in the earlier stage of cancer development. Due to mutational genetic instability in DNA causes the modulations in the signal transduction or even induce error in replication would efficiently reflects in induction or repression of specific transcription of the genes related to carcinogenesis. The recent investigation also described that the increased level of intracellular ROS in cancer cells possess a constant and chronic oxidative stress, and thereby it provides a favorable microenvironment for tumor progression. Further, the p53 tumor suppressor was found to be mutated and accounts for up to approximately 50% of all human cancers and is a major activator of antioxidant defense mechanisms. With respect to its apoptosis-inducing capacity, molecules that promote ROS production may be novel therapeutics, proposed to selectively target cancer cells by elevating ROS levels beyond a tolerable threshold to induce mitochondrial outer membrane permeabilization (MOMP) and cell death (Graham et al. 2010).

2.4 Flavonoid

From the ancient years, our folk medicine has been made of different and variety of naturally occurring plant products which later we call as nutraceuticals. Flavonoids are polyphenols produced as secondary byproducts of plants, which were

considered as main constituents of nutraceuticals. Hence, these flavonoids possess numerous pharmacological properties, and these polyphenols were consumed for hundreds of years as diet in form of fruits and vegetables; therefore these polyphenols were called as dietary flavonoids (Li et al. 2018). Chemically these flavonoids were characterized to have basic hydroxylated phenyl moieties with a number of phenolic rings and electron donation functional groups (Manach et al. 2004). Basically, these flavonoids are classified as polyphenols that are responsible for pigmentation in plants. Such polyphenols have a significant physiological function in plants to perform maturation of seeds, actively encounters biotic and abiotic stress of plants, maintains homeostasis of heat, freezing tolerance, act as a defense mechanism against pathogens and takes part in plants detoxification mechanism (Mierziak et al. 2014). Out of thousands of identified flavonoids, diphenyl propane skeleton (C6–C3–C6) with two phenyl ring and one heterocyclic ring form the basic structure of all the flavonoids (Kawser Hossain et al. 2016). Briefly, flavonoids are low molecular weight large group bioactive polyphenols with a basic structure of benzo-γ-pyrone and are widely found in a variety of plants. These flavonoids contain the number of hydroxyl group along with the catechol group at 3- and 5-positions at flavon ring that attributes its radical scavenging effect and antioxidant properties. Based on the structures, these flavonoids were classified into six major subclasses that can be listed as flavones, flavanones, flavanols, flavonols, isoflavones, and anthocyanins/anthocyanidins (Fig. 2.1). A large number of plant-derived bioactive polyphenols have been studied and evidently proved as efficient anti-carcinogenic agents. These flavonoids potentially block cancer development by interfering at multi-stages of carcinogenesis either at initiation, development, or progression of tumors; thereby these molecules have a significant inhibitory effect on proliferation and differentiation of cells and block angiogenesis and metastasis along with a decrease in tumor mass by induction of programmed cell death (Ramos 2007). Earlier research studies evidence that secondary metabolites include flavonoids which are phenolic in nature that possess several pharmacological activities which are structure-dependent (Mahomoodally et al. 2005). The specified quantity of hydroxylation, conjugations with other substitutional functional groups, and polymerization basically determines the structure and chemical nature of the flavonoids. It is well known that flavonoids were rich in hydroxyl groups and functionally act as scavengers to neutralize the free radicals and act as an antioxidant. It also has the capability to chelate metal ions. Previous studies evidenced that these flavonoids possess a chemoprotective effect against various degenerative and infectious diseases like cancer and cardiovascular diseases.

2.5 Functional Flavonoids as Antioxidants

Brief reviews from vast literature denoted that variety of flavonoids act as an antioxidant; in particular, it possesses reducing power to scavenge highly reactive radicals that are generated during human metabolic functions (Brunetti et al. 2013). Previous research investigations on antioxidant activity of these polyphenolic

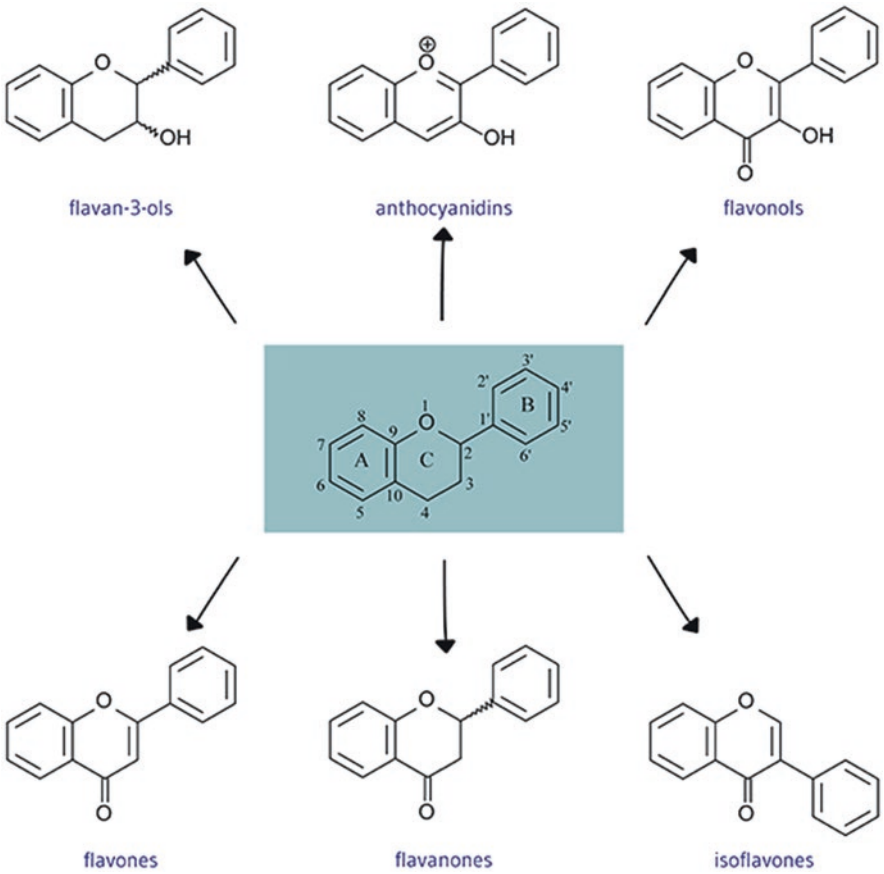

Fig. 2.1 Basic structure of flavonoids

compounds have shown that flavonoids efficiently inhibit specific enzymes like xanthine oxidase and protein kinase C which produce active superoxide radicals (Hanasaki et al. 1994). Also, these bioactive compounds inhibit a wide range of enzymes like cyclooxygenase, lipoxygenase, microsomal monooxygenase, etc. that involves in lipid peroxidation metabolism. An extensive report on the relation between the structure of free radicals and the respective flavonoids had revealed the radical scavenging mechanism and demonstrated its antioxidant potentials (Rice-Evans et al. 1996). Numerous in vivo study models pictured that dietary flavonoids readily scavenge free radicals in the stomach, and thereby they reduce ROS and act as primary antioxidant defense system (Papas 1998). Green medicines always get more attention toward researchers and are an important tool to prevent, reverse, or delay the carcinogenesis. Therefore, prevention could be a possible option for expensive treatments for existing disease. There have been many more compounds that are in a trial which have been declared as prospect chemopreventive agents. It

is well known that these bioactive compounds were commonly present in many food products, commercially available beverages, and many other dietary components. Consumption of these compounds directly or indirectly acts as inhibitory agents on cancer cell proliferation, and thereby these plant flavonoids in foods serve as preventive shield against human carcinogenesis implicated in the prevention of human carcinogenesis possibly through their inhibitory activities of cell proliferation or survival. Decades of research were interested to fix on the natural compounds that include any plant byproduct substances to unmask their anti-carcinogenic effect by tuning the level of intracellular lipid peroxidation and modulate the status of antioxidant that would be used as chemopreventive medicine in the treatment of colorectal cancer. The following are a short brief of few bioactive compounds which were recently studied for their anticancer activity against colorectal cancer.

2.5.1 Eriodictyol

Number of bioactive compounds that are in a trial which have been declared as prospect chemopreventive agents; one of the novel flavonoids recently studied was eriodictyol which comes under flavanone and has been proved to possess antioxidant effect by reducing the level of lipid peroxidation and oxidative stress. The flavonoid rich in citrus and lemons with chemical structure 5,7,3′,4′-tetrahydroxyflavanone was called eriodictyol, and it is naturally occurring in citrus fruits in glycoside form. Eriodictyol was previously considered as vitamin P that was found to be essential in normal small blood vessels (Horowitz 1958). Eriodictyol is an isomer of kaempferol and has been isolated from yerba santa (*Eriodictyon californicum*). Other major sources of eriodictyol were identified as citrus fruits including lemon, lime, and sour orange and green leaves like peppermint. A number of studies had reported few pharmacological properties such as antioxidant, anti-inflammatory, and anticancer activities. Hence the structural chemistry of eriodictyol consists of free electron-donating hydroxyl moieties, which would impact on potent free radicals' scavenging activities which in turn reduces the oxidative stress. Previous experiments on supplementation of eriodictyol to rodent model bearing DMH-induced colon cancer had reported with a significant anti-carcinogenic efficacy against colon cancer models (Fig. 2.2). The study had described that eriodictyol primarily alters antioxidant status, gut microbial enzyme activity, and lipid oxidation status in cancer-bearing rats. Prolonged treatment with this flavonoid had intensively reduced pre-neoplastic lesions along with the decrease in aberrant crypt numbers that was reported. β-Glucuronidase suppressor would effectively reduce ACF formation that was early reported in past decades (Takada et al. 1982). Cordially another study also evidently pointed out that chronic treatment of eriodictyol had highly influenced the reduction of β-glucuronidase activity in DMH-induced colon cancer rat models. It is likely to be believed that oxidative stress and lipid peroxidation induce cancer cell initiation and proliferation (Peluso et al. 2011). In connection, extensive study with eriodictyol supplementation to colon cancer-bearing rats showed a notable decrease in lipid peroxidation and pro-oxidant status; thereby colon cancer

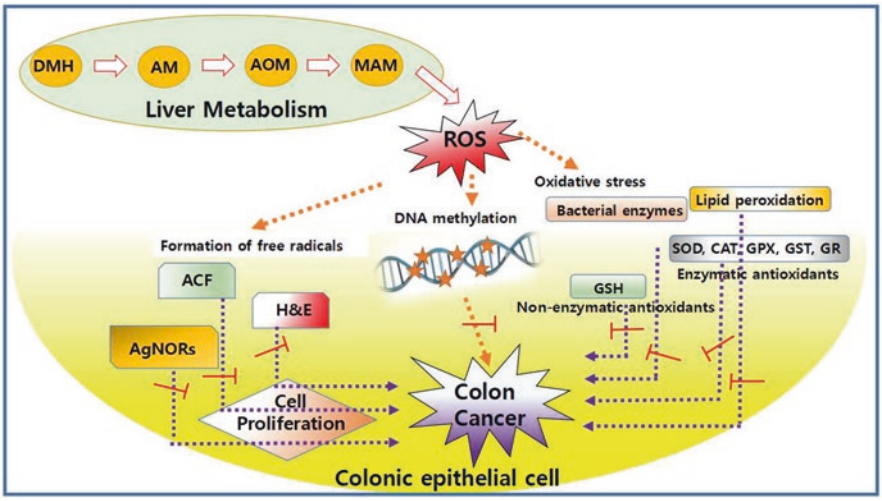

Fig. 2.2 Graphical representation of eriodictyol's protective mechanism against DMH-induced colorectal cancer models

progression and severity were highly reduced (Mariyappan et al. 2017). Highlights of eriodictyol on gut enzyme activity were reported as considerable inhibition of sulfo-conjugations; thereby nitroreductase sulfatase enzyme activities were thought to be decreased, and on the other hand it is well studied that high number of cancer was reported on increased activity of gut sulfatase and nitroreductase enzymes. As a pictorial proof, eriodictyol's anti-carcinogenic effect was reported with the histological examination on eriodictyol-supplemented rat models, and the study had pictured the architecture of the colon cancer cells after treatment, and the abnormal changes were highly reduced with decreased dysplastic cells in the colon tissue of flavonoid-treated rats. As a whole, this review about eriodictyol had briefly elaborated the mechanism of antioxidant potential and anti-carcinogenic effect against cancer models.

2.5.2 Umbelliferone

Umbelliferone had been a known and well-studied coumarin, a flavonoid with benzopyrone in nature, and was widely spread in common plants and herbs like parsley sanicle, fennel, celery, giant hogweed, cumin, and asafoetida (Mazimba 2017). This particular bioactive coumarin compound was reported to possess an effective antioxidant role; hence it has been derived from plant polyphenols and consumed as diet in form of fruits and vegetables from ancient days (Hoult and Payá 1996). Here we concise a summarized view on antioxidant potential and anti-carcinogenic effect of pharmacologically active 7-hydroxycoumarin, i.e., umbelliferone. A recent research was highly considered that the use of potentially active

phyto-flavonoids is a novel and efficient method to treat and eliminate colon cancer as illustrated in (Muthu et al. 2016). These bioactive compounds that include umbelliferone have been found to modulate the various signaling pathways; thereby it considerably inhibits the pathogenesis of colorectal cancer, and it is designated as a novel anticancer agent. One of the previous studies in our team had efficiently proved that umbelliferone had stiffly and strongly inhibited CRC in DMH-induced colon cancer rat models (Muthu et al. 2016). It is well studied that DMH induction in rats generates a large amount of ROS and causes genetic mutation in colon genes which would be resulted in an initial increase in aberrant crypt formation and later the pathogenesis that lead to colon epithelial carcinoma (Sengottuvelan et al. 2006). Earlier study on rats had reported that intraperitoneal administration of umbelliferone to CRC-bearing rats significantly inhibited and reduced the formation of a specified number of the aberrant crypt in colon epithelial at an early stage of colon cancer. It is to be highlighted that the study also confirmed with the histological pictures of reduced ACF in umbelliferone-treated CRC rat models. Further, the same study had stated that umbelliferone has efficacy to arrest cancer cell proliferation and the AgNOR quantification with image tools that had indexed the reduced cell activation in umbelliferone-treated to CRC-bearing rats (Sirri et al. 1995). Subsequently, mast cells provide a considerable microenvironment for cancer cells to proliferate and survive by means of increased angiogenesis (Oldford and Marshall 2015).

Further reports from previous literature had stated that umbelliferone-treated to CRC-bearing rats have a significant decrease in a number of mast cells, and it clearly indexed the anti-inflammatory effect of umbelliferone in carcinogenesis models. Extensive study reports of primary inflammatory markers like tumor necrosis factor-alpha (TNF-α), interleukin-1, *cyclooxygenase* (*COX*), and iNOS that were known to be activated by ROS on CRC models had indicated that umbelliferone significantly had changed the microenvironment of tumor cells by downregulating the expression of intestinal inflammatory marker in cancer-bearing rats (Takahashi et al. 2015). Thereby it has been evidently shown that umbelliferone modulates NF-kB signaling pathway.

Also, umbelliferone had been reported to induce apoptosis and DNA fragmentation by activation of Bax-dependent caspase pathway of apoptosis in CRC rats (Fig. 2.3). As a whole, this brief review about umbelliferone against CRC has clearly pictured the potential use of this particular flavonoid to eliminate colon cancer. Further intensive preclinical and clinical studies on cancer models would be helpful to unravel its complete mechanism of action and would be made commercially available for cancer patients.

2.5.3 Luteolin

Luteolin was classified under flavones of flavonoids based on its chemical structure. Chemically its architecture is of 3′,4′,5,7-tetrahydroxyflavone and was found widespread in most of the plants. Luteolin structure is composed of A and B, two benzene ring, one C ring with oxygen, and a double bond at 2–3 along with readily

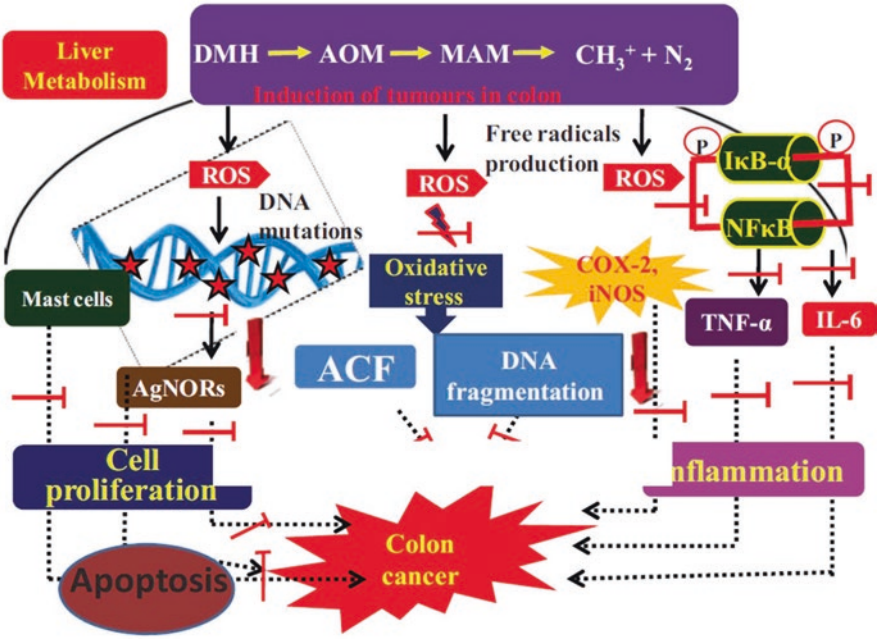

Fig. 2.3 Schematic representation of umbelliferone against DMH-induced colon cancer

reducing several hydroxyl groups at 5-, 7-, 3′-, and 4′-positions as shown in (Ross and Kasum 2002). Abundant and naturally occurring sources of luteolin supplementation that were available such as parsley, onion leaves, broccoli, celery, cabbages, chrysanthemum flowers, carrots, peppers, and peanuts were listed (Pandurangan and Esa 2014). Hence it consists of a large number of reducing groups, and it can readily donate an electron to free radicals and scavenge generated ROS. Further literature studies have cumulatively accounted pharmacological properties such like cardio-protective (Madhesh and Vaiyapuri 2013), antioxidant (Ashokkumar and Sudhandiran 2008), anticancer (Manju and Nalini 2005) (Manju), and anti-inflammatory activities (Nishitani et al. 2013). Previous investigations had stated that luteolin possesses significant antiproliferative effect against various cancers that include breast, liver, prostate, esophageal, and lung cancers; thereby it plays a key role as strong chemoprotective agent (Pandurangan and Esa 2014). Any abnormal increase in neoplastic cells due to the absence of apoptosis leads to tumor mass progression with increased cell differentiates, angiogenesis, oxidative stress due to over metabolic process and resulted with metastasis of cancer. Overproduction of ROS due to increased metabolism is not only the factor for cancer progression; lack of sufficient antioxidant defense to scavenge the radicals generated by cancer cell plays a vital role in carcinogenesis (Cheng et al. 2012). Therefore normal cells require endogenous and supplemented antioxidants to fight against the oxidative stress generated by cancer cells. Hence, luteolin possesses more electron donor

functional groups, and it serves as a rapid antioxidant to scavenge the unstable reactive free radicals to protect the cells from intracellular ROS lesions. In particular, luteolin induces cell cycle arrest and programmed cell death; thereby it efficiently blocks the invasion of cancer cells. Earlier research studies on cancer therapy had explained about the apoptosis mechanism and it was initiated by death receptors and sub-sequential activation of mitochondrial-dependent caspase pathway (Ma et al. 2018). Large number of bioactive compound has a potential chemoprotective effect to induce programmed cell death in cancer cells that were evaluated by a number of investigators in past decades, and also the previous study reports the anticancer efficacy of luteolin against human colon cancer cells that were sequenced by induced cell cycle arrest and followed with activation of caspase-dependent intrinsic apoptotic pathway (Pandurangan and Esa 2014). Pathogenesis of colon cancer in DMH rats involves lipid peroxidation to form active malondialdehyde, conjugated dienes, and lipid hydroperoxides, which causes considerable damage in cells. In our previous study using DMH-induced rate models, we had evidently pictured and elaborated mechanism of action on how the target flavonoid luteolin had significantly reduced the tumor cell number mediated by a decrease in lipid peroxidation and inversely increased endogenous enzyme-dependent antioxidant mechanism. For the past decades level of glycoprotein in serum was considered as one of the hallmark markers to assess cancer, hence this glycoprotein plays a key role in cell-cell recognition, cellular adhesion, binding, and cellular transport, during rapid proliferation and differentiation of cancer cells the level of this specific protein would be elevated and its server as valuable marker carcinogenic process. Luteolin-administered DMH rats found to have a lower level of these glycoproteins were reported (Pandurangan et al. 2012). Earlier literature reports on luteolin had stated to induce cell shrinkage followed by chromatin condensation and finally leads to DNA fragmentation, which was said to be the hallmark of apoptosis in colon cancer cells. Activation of effector caspases and Bax/Bcl2 ratio critically determine the fate of the cancer cell to induce apoptosis. This was followed by the successive release of mitochondrial cytochrome C conform the activation of mitochondrial-dependent intrinsic apoptosis and this hypothesis was tested with various cancer cell against luteolin treatment was reported (Attoub et al. 2011; Pandurangan and Ganapsam 2013). Use of such naturally occurring phytocompounds in the prevention of colon cancer was expected to be safe and avoid the adverse effect of anticancer drugs like synthetic chemotherapeutic compounds. This chapter also summarizes the ample of research evidence from the past study reports to show the chemopreventive potential of bioactive flavonoids in the prevention of colon carcinoma.

2.6 Conclusions

Consumption of dietary flavonoids in a consistent manner and an adequate amount has been proven to be a significant nutritional therapy to prevent and lower the risk of colorectal cancer, which could be implicated to enhance for cancer treatments. This chapter insensitively discussed about the flavonoids as chemopreventive drugs

to treat cancer, and also elaborated the primary mechanisms of action of such bioactive flavonoids on chemically/carcinogen-induced oxidative stress. Efficacy of flavonoids to reduce the oxidative stress by scavenging ROS is considered as a principal factor to act as antioxidant and anti-inflammatory properties. Hence, colon cancer is a chronic disease, and its progression was brought by continuous bowel inflammation caused by oxidative stress and various other factors. Supplementation of flavonoids potentially prevents the inflammation, thus controlling colon cancer progression and invasion. Further, it modulate the expression of oncogenic and apoptotic proteins in cancer models, thereby activating Bax/Bcl2-mediated mitochondrial apoptotic pathway. A number of recent studies also have pinpointed the synergetic effects of flavonoids along with other anticancer drugs. Hence, the present review concludes that flavonoids have a significant anticancer potential against colon cancer, and it can be used as drug/supplements to prevent inflammation, and it helps to eradicate cancer diseases. However, the prospective cohort preclinical and clinical studies on flavonoids would help to better understand other mechanisms of dietary flavonoids for improved implication in the treatment of a number of cancer diseases.

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Plant Neoflavonoids: Chemical Structures and Biological Functions

3

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Abstract

Neoflavonoids (NFs) constitute a remarkable group of naturally occurring flavonoids with C6-C3-C6 (4-phenylcoumarin) carbon skeleton. NFs are not often found in food and edible plants, but widely distributed in different plant families, including Fabaceae, Clusiaceae, Leguminosae, Rubiaceae, Passifloraceae, Thelypteridaceae, and Polypodiaceae. They are commonly being identified in various plants, belonging to *Dalbergia* genus. Because of dalbergin and other NFs, *Dalbergia* species are having medicinal importance, and more than 60 NFs are being isolated and identified so far from this genus. Depending on the pattern of substitution and sources, they are categorized into four groups, namely 4-arylcoumarins (neoflavones), 4-arylchromanes, dalbergiones, and dalbergiquinols. These compounds have been found to display several health beneficial effects. They have wide-ranging therapeutic properties, such as anti-osteoporosis, anti-inflammatory, antimicrobial, antiplasmodial, anti-androgen, anti-allergic, antioxidant, antifungal, antidiabetic, and anticancer activities. Additionally, NFs of various medicinally valued plants are being employed as herbal formulations in the traditional systems of medicine like Ayurveda, Unani, Chinese, etc., around the world. The aim of this chapter is to discuss different structures and biological functions of NFs isolated from different plant species with a particular focus on the recent therapeutic approaches.

Keywords

Bioactive compounds · Chromatography · Extraction techniques · HPLC · Phytomedicine

3.1 Introduction

Neoflavonoids (NFs) constitute a remarkable group of naturally occurring flavonoids with C6–C3–C6 (4-phenylcoumarin) carbon skeleton. NFs are not often found in food and edible plants, but widely distributed in approximately 58 plants belonging to different families, including Fabaceae, Clusiaceae, Leguminosae, Rubiaceae, Passifloraceae, Thelypteridaceae, and Polypodiaceae. They are commonly being identified in various plants of *Dalbergia* genus (Garazd et al. 2003). Dalbergin, isolated from different species of *Dalbergia* is the most common and largely occurring neoflavone in plants. Therefore, this genus is considered to be of high interest among researchers, and beyond 60 NFs are being isolated and identified so far (Kumar et al. 2014). In 1951, calophyllolide was the first neoflavone isolated from the extract of *Calophyllum inophyllum* seeds. Likewise, dalbergin compound was firstly isolated from *D. latifolia* (Fig. 3.1). Also, calophyllolide occurs in the barks and timbers of an endemic tree species, occurring in Sri Lanka, i.e., *Mesua thwaitesii* (Garazd et al. 2003). NFs occur as glycosides, aglycones, and methylated byproducts. The basic configuration differs from other flavonoid

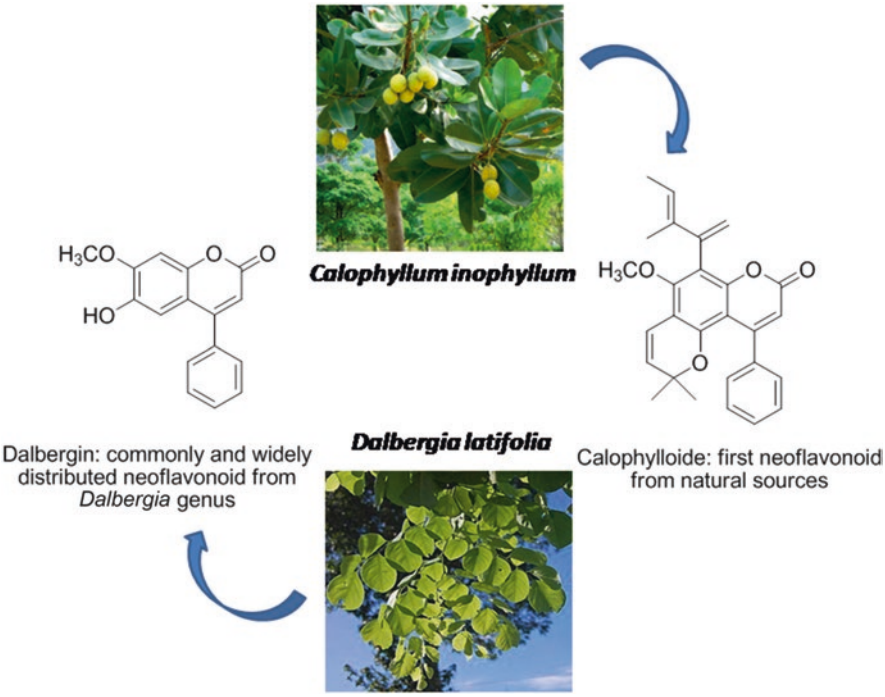


Fig. 3.1 *Calophyllum inophyllum* tree seeds and *Dalbergia latifolia* leaves showing the presence of calophyllolide and dalbergin compounds, respectively

structures via the presence of aryl group at 4-position. They are often hydroxylated at different positions. In glycosides, the presence of carbohydrates can be D-glucose, L-rhamnose, galactose, glucorhamnose, or arabinose (Panche et al. 2016). NFs have been found to display several health beneficial effects, and therefore have wide-ranging biological activities, including anti-osteoporosis, anti-inflammatory, anti-microbial, antiplasmodial, anti-androgen, anti-allergic, antioxidant, antifungal, antidiabetic, and anticancer activities (Luqman et al. 2012). Additionally, NFs of various medicinally valued herbs are being utilized in herbal formulations of the traditional systems of medicine like Ayurveda, Unani, and Chinese, etc., around the world. The aim of this chapter is to discuss different structures and biological functions of NFs isolated from different plant species with a particular focus on the recent therapeutic approaches.

3.2 Classification of Neoflavonoids

Depending on the pattern of substitution and sources, neoflavonoids have been classified into two main groups, i.e., the 4-phenylcoumarins (dalbergin group) and diphenyl allyl compounds (latifolin group) (Fig. 3.2).

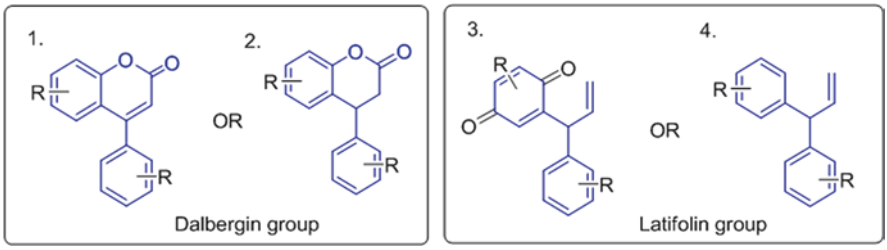


Fig. 3.2 Basic skeletons of different classes of neoflavonoids

3.2.1 Dalbergin Group

4-Phenylcoumarin categories of neoflavonoids have been sequestered from different parts of diverse plants. They include compounds like dalnigrin (**1**) from *D. nigra*, melanettin (**2**) from *D. odorifera*, melannein (**3**) from *Pterocarpus santalinus*, 3′-hydroxymelanettin (**4**) from *D. odorifera*, 6-hydroxy-4-(4-hydroxyphenyl)-7-methoxy-2*H*-1-benzopyran-2-one (**5**) from *D. stevensonii*, dalbergin (**6**) from *D. latifolia*, and methyldalbergin (**7**), isodalbergin (**8**), and nordalbergin (**9**) from *D. sissoo* plant (Fig. 3.3) (Donnelly et al. 1968, 1973; Mukerjee et al. 1971a, b; Chan et al. 1997; Ragab et al. 2006, Kite et al. 2010; Zhaoa et al. 2013; Kumar et al. 2014).

Other types of 4-phenylcoumarin includes kuhlmannin (**10**) from *D. nigra*, 5,6,7,4′-tetramethoxy-4-phenylcoumarin (**11**) and 5-hydroxy-7-methoxy-4-(3,4-dihydroxyphenyl)-2*H*-1-benzopyran-2-one (**12**) from *Coutarea hexandra*, 5,7,4′-trimethoxy-4-phenylcoumarin (**13**) from *Chiococca alba*, 3′,7-dihydroxy-4′,5-dimethoxy-4-phenylcoumarin (**14**) from *D. volubilis*, 3′,5-dihydroxy-4′,7-dimethoxy-4-phenylcoumarin (**15**) from *Exostema acuminatum*, nivetin (**16**) from *Echinops niveus*, nivegin (**17**) from *Glycyrrhiza glabra*, 2′,5′,5-trihydroxy-7-methoxy-4-phenylcoumarin (**18**) from *C. latiflora*, 4-(2,5-dihydroxyphenyl)-5-hydroxy-7-methoxy-2*H*-1-benzopyran-2-one (**19**) from *C. hexandra*, 9,10-dihydroxy-5-methoxy-2*H*-pyrano[2,3,4-*kl*]xanthen-2-one (**20**) from *C. latiflora*, and 5,7-dihydroxy-4-phenylcoumarin (**21**) from *Passiflora serratodigitata* along with glycosides as 7-(*β*-D-glucopyranosyloxy)-5-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one (**22**) from *P. serratodigitata* and 4-(3,4-dihydroxyphenyl)-5-(O-β-D-galactopyranosyl)-7-methoxycoumarin (**23**) from *E. caribaeum* as well as biglycosides like 5-hydroxy-4-phenyl-7-4-O-β-D-glucopyranosyl-*β*-D-galactopyranosyl)oxy]2*H*-1-benzopyran-2-one (**24**) from *Tephrosia purpurea* (Ulubelen et al. 1982; Reher et al. 1983; Chawla and Mittal 1984; Reher and Kraus 1984; Monache et al. 1987, 1989; Singh and Pandey 1990; Kusano et al. 1991; Donnelly and Boland 1995; Saxena and Choubey 1997; Ito et al. 2000; Kite et al. 2010).

Some of the 5,7-disubstituted coumarin derivatives include 3,4-dihydro-5-hydroxy-7-methoxy-4-4-methoxyphenyl)2*H*-1-benzopyran-2-one (**25**) from *Polygonumperfoliatum*, 3,4-dihydro-5-hydroxy-4-4-hydroxyphenyl)7-methoxy-2*H*-1-benzopyran-2-one (**26**) and 3,4-dihydro-5,7-dihydroxy-4-4-methoxyphenyl)2*H*-1-benzo

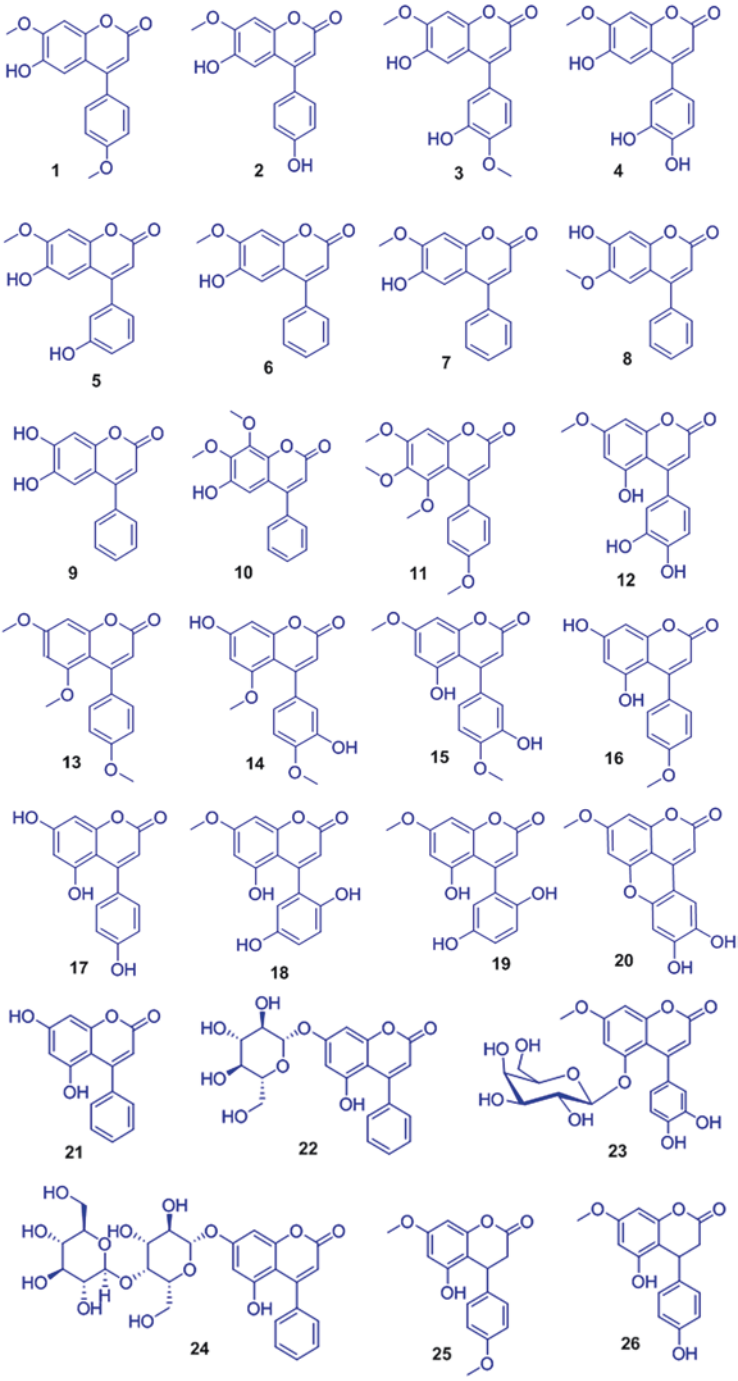
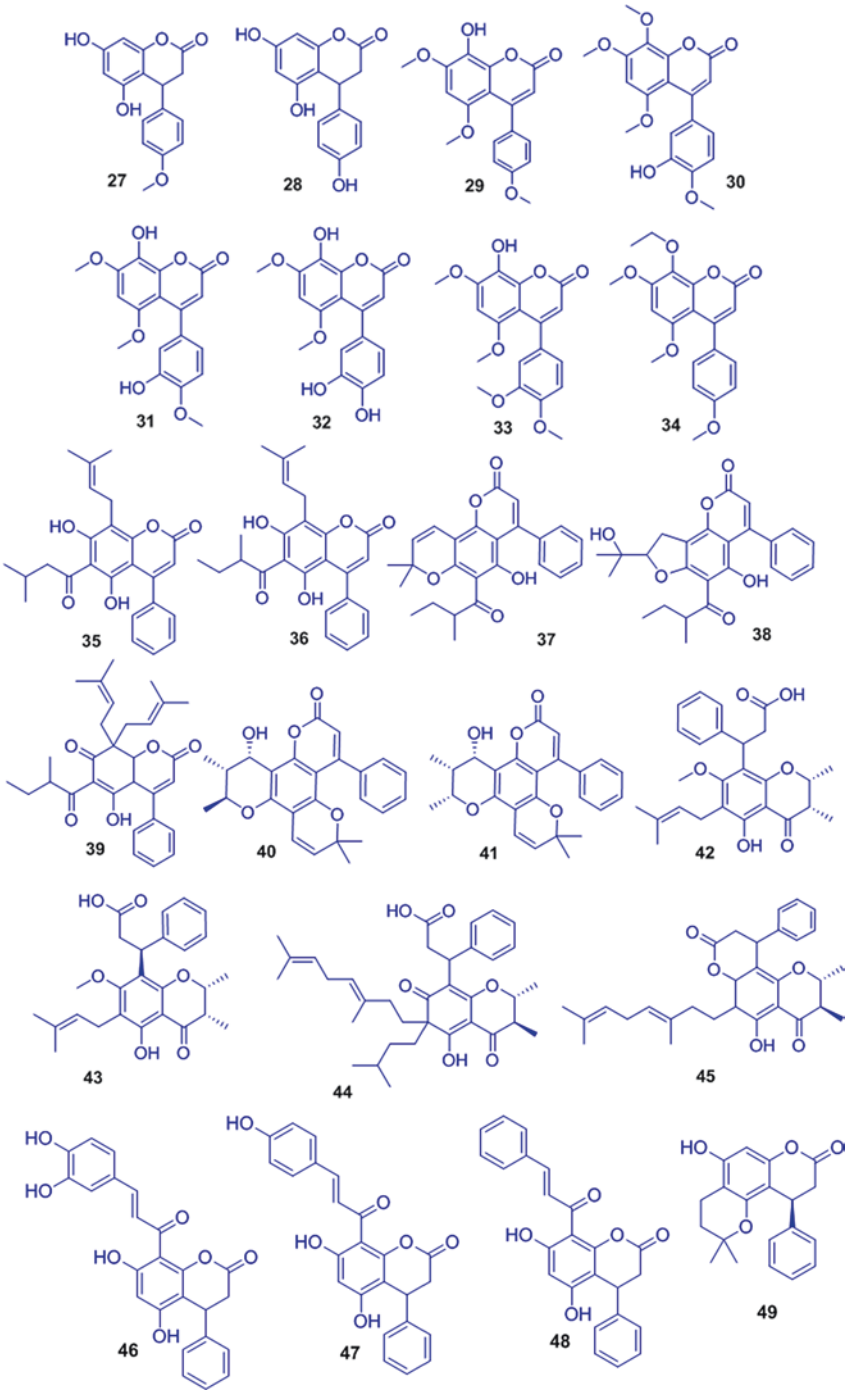


Fig. 3.3 Chemical structures of neoflavonoids



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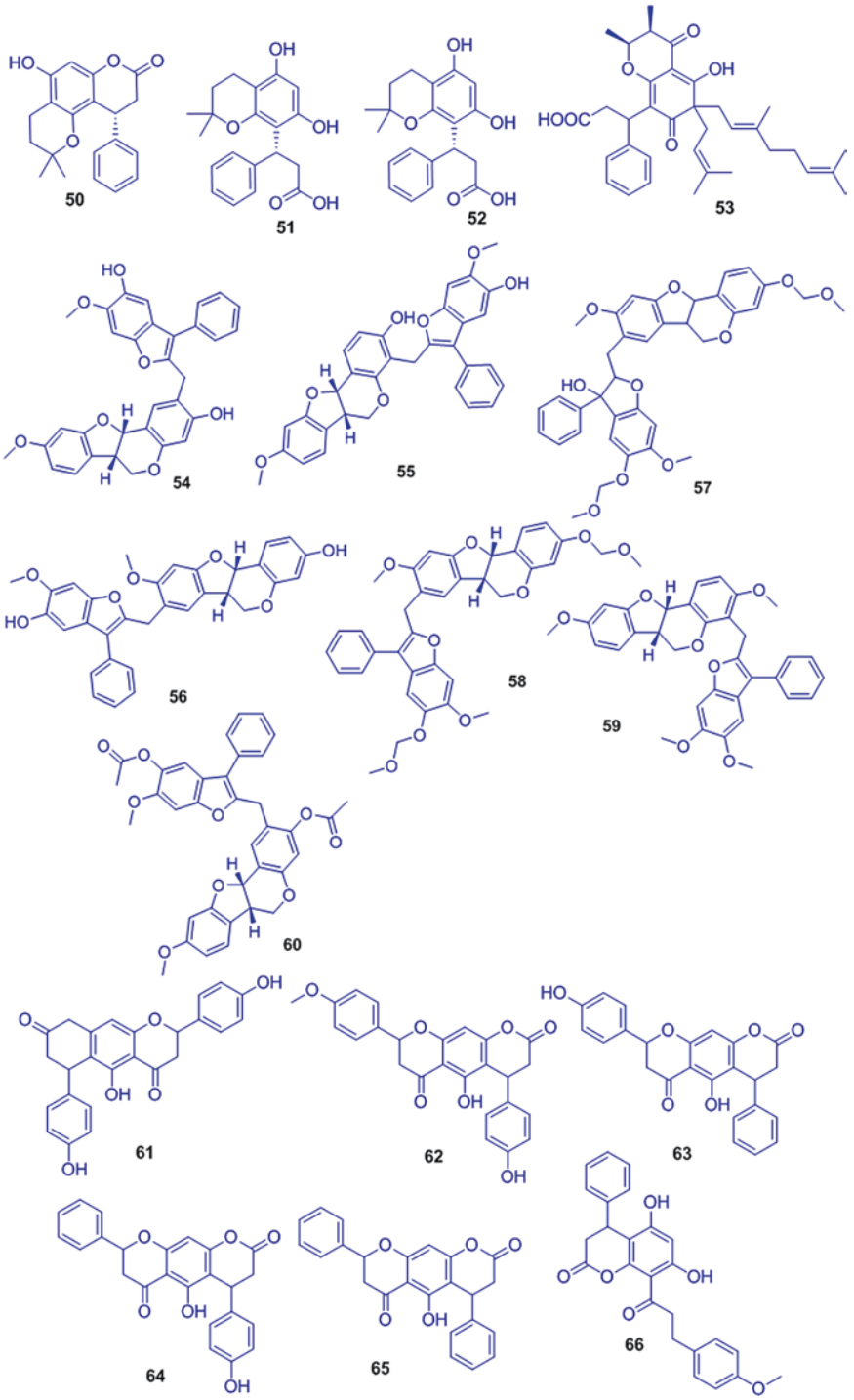

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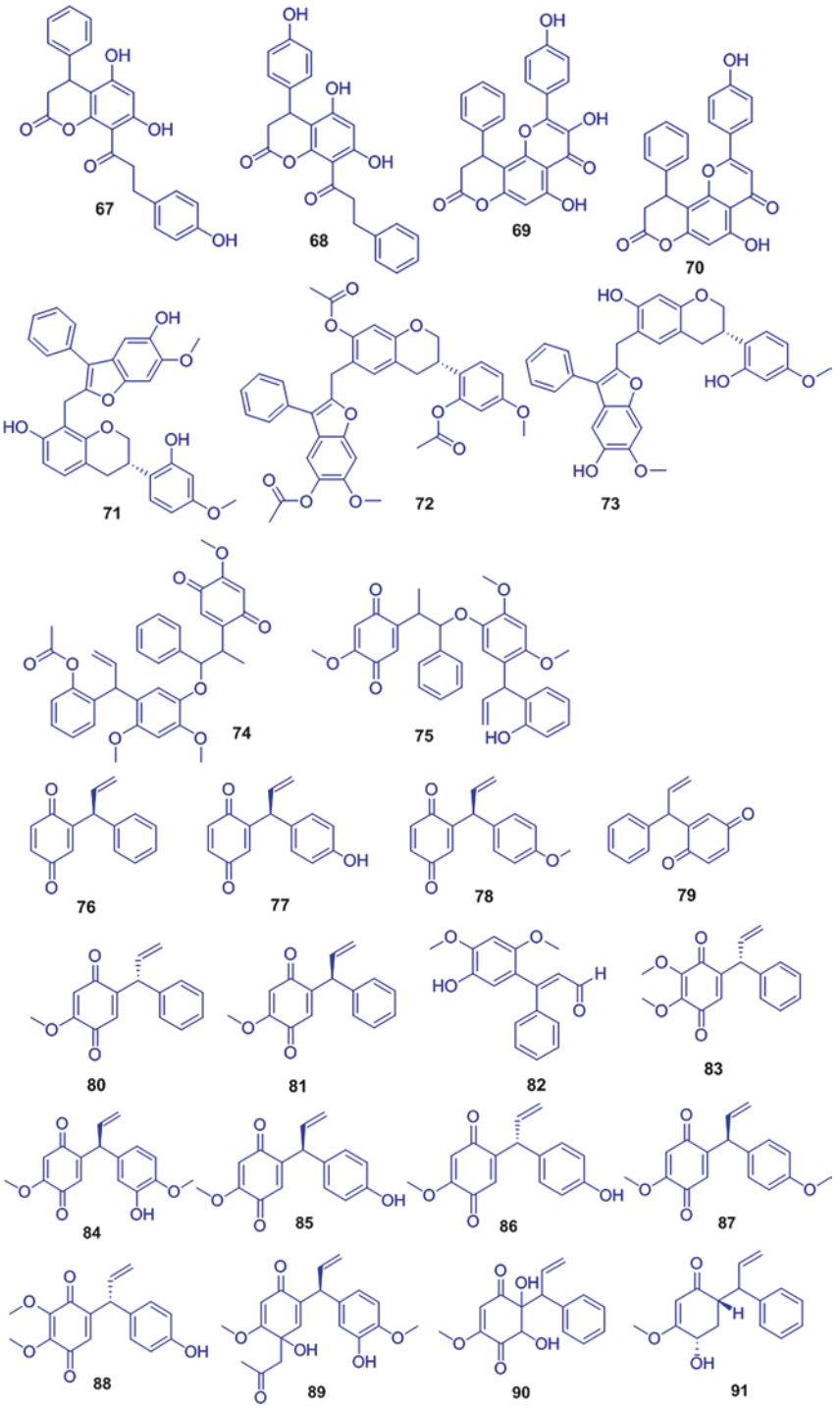

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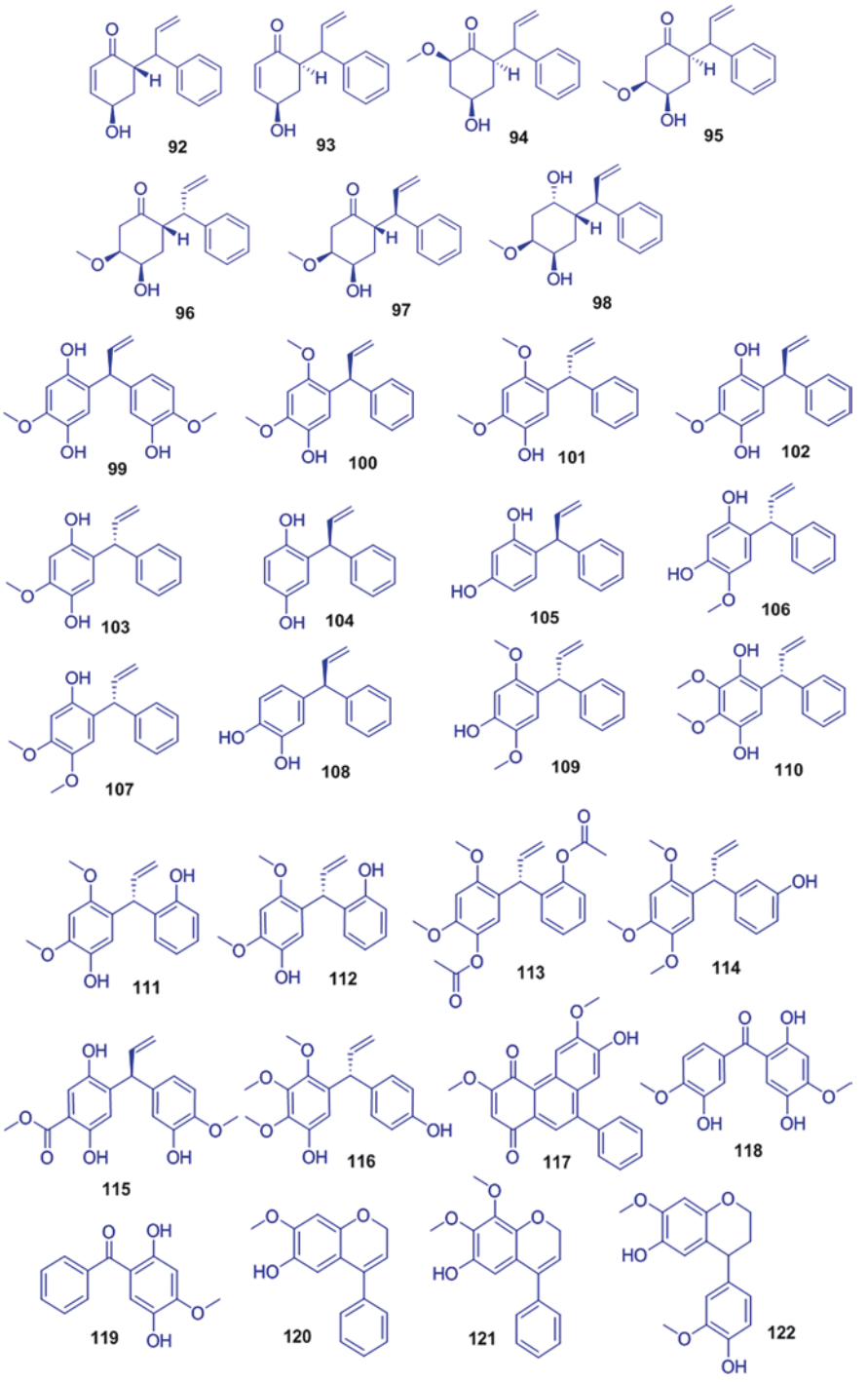

Fig. 3.3 (continued)

pyran-2-one (**27**) from *Dalbergia nigra* and 3,4-dihydro-5,7-dihydroxy-4-4-hydroxyphenyl)2*H*-1-benzopyran-2-one (**28**) from *P. perfoliatum*, have been isolated. On the basis of 5-, 7-, and 8-position, 4-phenylcoumarin includes exostemin (**29**), from *C. alba*, and 4-3-hydroxy-4-methoxyphenyl)-5,7,8-trimethoxy-2*H*-1-benzopyran-2-one (**30**), 3′,8-dihydroxy-4′,5,7-trimethoxy-4-phenylcoumarin (**31**), 4-(3,4-dihydroxyphenyl)-8-hydroxy-5,7-dimethoxy-2*H*-1-benzopyran-2-one (**32**), 8-hydroxy-5,7,3′,4′-tetramethoxy-4-phenylcoumarin (**33**), and 4′,5,7,8,-tetramethoxy-4-phenylcoumarin (**34**) from *C. hexandra* (D'Agostino et al. 1989; D'Agostino et al. 1990; Donnelly and Boland 1995; Sun and Sneden 1999; Kite et al. 2010).

Another type of 4-phenylcoumarin is tetrasubstituted, which includes mammea A (**35**), MAB 1 (**36**), MAB 5 (**37**), and MAB 3 (**38**) from *Mammea africana* and calofloride (**39**) from *C. verticillatum* and (−)-*trans*-dihydroinophyllolide (**40**) and (+)-*cis*-dihydroinophyllolide (**41**) from *C. mooni* along with some open-chain compounds like (2*R*,3*S*)-2,3-dimethyl-5-hydroxy-6-(3-methylbut-2-enyl)-7-methoxy-8-(2-carboxyl-1-phenylethyl)-2,3-dihydrobenzopyran (**42**) and (*βS*,2*R*,3*S*)-3,4-dihydro-5-hydroxy-7-methoxy-2,3-dimethyl-6-(3-methyl-2-buten-1-yl)-4-oxo-*β*-phenyl-2*H*-1-benzopyran-8-propanoic acid (**43**) from *C. inophylloide* and calozeylanic acid (**44**) as well as closed-chain calozeylanic lactone (**45**) that were isolated from *C. lankaensis* and *C. thwaitesii* (Carpenter et al. 1970, Ramiandrasoa et al. 1983, Bandara et al. 1986, Ravelonjato et al. 1987, Goh et al. 1992, 1993, Dharmaratne et al. 1984).

The Neoflavonoids having cinnamoyl moeity were isolated from *Pityrogramma trifoliata* plant, for example, (*E*)-8-(3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl)-3,4-dihydro-5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one (**46**), (*E*)-3,4-dihydro-5,7-dihydroxy-8-(3-(4-hydroxyphenyl)-1-oxo-2-propenyl)-4-phenyl-2*H*-1-benzopyran-2-one (**47**), and (*E*)-3,4-dihydro-5,7-dihydroxy-8-(1-oxo-3-phenyl-2-propenyl)-4-phenyl-2*H*-1-benzopyran-2-one (**48**). Some 5,6,7-trisubstituted 4-phenylcoumarin like isothwaitesic lactone (**49**) and thwaitesic lactone (**50**) and some open-chain 5,6,7-substituted 4-phenylcoumarin such as thwaitesic acid (**51**) and isothwaitesic acid (**52**) have been isolated from *C. lankaensis* and *C. thwaitesii* plants. Calozeylanic acid (**53**) was also isolated from *C. walker* (Dharmaratne et al. 1984).

Another type of skeleton is pterocarpan-neoflavonoid which includes daljanelin A (**54**), daljanelin B (**55**), daljanelin C (**56**), 2-((6a,11a-dihydro-9-methoxy-3-(methoxymethoxy)-6*H*-benzofuro(3,2-*c*)(1)benzopyran-8-yl)methyl)-2,3-dihydro-6-methoxy-5-(methoxymethoxy)-3-phenyl-3-benzofuranol (**57**), (6a*S*-*cis*)-6a,11a-dihydro-9-methoxy-3-(methoxymethoxy)-8-((6-methoxy-5-(methoxymethoxy)-3-phenyl-2-benzofuranyl)methyl)-6*H*-benzofuro(3,2-*c*)(1)benzopyran (**58**), daljanelin B di-*O*-methyl ether (**59**), and daljanelin A diacetate (**60**). All these compounds were isolated from *D. nitidula* (Ferreira et al. 1995).

Many calomenols (E, G, H, I, J) (**61**–**65**) and calomenols (A, B, C, D, and F) (**66**–**70**) have been isolated from *P. calomelanos*. Calomenols (H, D, F) were also isolated from *P. tartarea*. Some isoflavonoid-neoflavonoid such as daljanelin D (**71**), (3*S*)-3-(2-(acetyloxy)-4-methoxyphenyl)-6-((5-(acetyloxy)-6-methoxy-3-phenyl-2-benzofuranyl)methyl)-3,4-dihydro-*H*-1-benzopyran-7-ol-7-acetate

(**72**), and (3*S*)-3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-6-((5-hydroxy-6-methoxy-3-phenyl-2-benzofuranyl)methyl)-1 benzopyran-7-ol (**73**) from *D. nitidula* also have been isolated. 2-(2-(5-(1-(2-(Acetyloxy)phenyl)-2-propenyl)-2,4-dimethoxyphenoxy)-1-methyl-2-phenylethyl)-5-methoxy-2,5-cyclohexadiene-1,4-dione (**74**) and 2-(2-(5-(1-(2-hydroxyphenyl)-2-propenyl)-2,4-dimethoxyphenoxy)-1-methyl-2-phenylethyl)-5-methoxy-2,5-cyclohexadiene-1,4-dione (**75**), a class of binary neoflavonoids, have been isolated from *D. latifolia* (Donnelly et al. 1981; Iinuma et al. 1994; Donnelly and Boland 1995; Ferreira et al. 1995; Bekker et al. 2002).

3.2.2 Latifolin Group

The second class of neoflavonoids is diphenyl allyl compounds (latifolin group) an open-chain neoflavonoid which includes so many compounds having different activities. These compounds were named as (*S*)-dalbergione (**76**), from *D. sissoo*; (*S*)-4′-hydroxydalbergione (**77**), from *D. nigra*; (*S*)-4′-methoxydalbergione (**78**), from *Dalbergia louvelii*; 2,3,5-trimethoxy-1,4-benzoquinone (**79**) from *rosewood*; and (*R*)-4-methoxydalbergione (**80**) from *D. sissoo* and *D. odorifera*. The enantiomer of **81** named as (*S*)-4-methoxydalbergione and dalsissooal (**82**) were isolated from *D. melanoxylon, D. inundata, D. riparia* and *D. sissoo* respectively. In addition, some different types of neoflavanoids namely (*R*)-3,4-Dimethoxydalbergione (**83**) from *Machaerium kuhlmannii*; (*S*)-3′-hydroxy-4,4′-dimethoxydalbergione (**84**) from *P. santalinus*; (*S*)-4′-hydroxy-4-methoxydalbergione (**85**) from *M. miscolobium*; (*R*)-4′-hydroxy-4-methoxydalbergione (**86**) from *D. odorifera*; (*S*)-4,4′-dimethoxydalbergione (**87**) from *Galerina marginata*; (*R*)-4′-hydroxy-3,4-dimethoxydalbergione (**88**) from *M. nictitans*; pterolinus Hb (**89**) from *P. santalinus*; 5,6-dihydroxy-2-methoxy-5-(1-phenylallyl)cyclohex-2-ene-1,4-dione (**90**), (4*S*,6*S*)-4-hydroxy-3-methoxy-6-(1-phenyl-2-propen-1-yl)-2-cyclohexen-1-one (**91**), and (4*R*,6*S*)-4-hydroxy-6-(1-phenyl-2-propen-1-yl)-2-cyclohexen-1-one (**92**) from *D. sissoo*; and (4*R*,6*R*)-4-hydroxy-6-(1-phenyl-2-propenyl)-2-cyclohexen-1-one (**93**) from *Nepalese propolis* have been isolated (Eyton et al. 1962; Braz et al. 1973; Leita de Almeida and Gottlieb 1974; Donnelly et al. 1975; Ollis et al. 1978, Gregson et al. 1978a, b; Imamura et al. 1982; Shirota et al. 2003; Awale et al. 2005; An et al. 2008; Shrestha et al. 2008; Wu et al. 2011; Kumar et al. 2014).

The other open-chain neoflavonoids are (2*R*,4*R*,6*R*)-4-hydroxy-2-methoxy-6-(1-phenyl-2-propen-1-yl)-cyclohexanone (**94**), (2*R*,4*R*,5*S*)-4-hydroxy-5-methoxy-2-(1-phenyl-2-propen-1-yl)-cyclohexanone (**95**), (2*S*,4*R*,5*S*)-4-hydroxy-5-methoxy-2-((1*S*)-1-phenyl-2-propenyl))cyclohexanone (**96**), (2*S*,4*R*,5*S*)-4-hydroxy-5-methoxy-2-((1*R*)-1-phenyl-2-propen-1-yl)-cyclohexanone (**97**), and (1*R*,2*S*,4*S*,5*S*)-2-methoxy-5-((1*R*)-1-phenyl-2-propen-1-yl)-1,4-cyclohexanediol (**98**) which have been isolated from *N. propolis*. Moreover, neoflavonoids showing biphenyl arrangement had been isolated and identified including pterolinus G (**99**) from *P. santalinus*, (7*S*)-dalbergiphenol (**100**) from *D. latifolia*, (*R*)-dalbergiphenol (**101**) and

(*S*)-4-methoxydalbergiquinol (**102**) from *D. sissoo*, (*R*)-4-methoxydalbergiquinol (**103**) from *D. sissoo*, and (*S*)-2-(1-phenylallyl)-benzene-1,4-diol (**104**) and 4-((1*S*)-1-phenyl-2-propenyl)-1,3-benzenediol (**105**) from *D. sissoo* (Gregson et al. 1978a, b; Awale et al. 2005; Sekine et al. 2009a, b; Wu et al. 2011; Shrestha et al. 2008; Kumar et al. 2014).

Another series of neoflavonoids, including 4-methoxy-6-((1*R*)-1-phenyl-2-propen-1-yl)-1,3-benzenediol (**106**) and 4,5-dimethoxy-2-((1*R*)-1-phenyl-2-propen-1-yl)-phenol (**107**) from *D. spinosa*, 4-((1*S*)-1-phenyl-2-propen-1-yl)-1,2-benzenediol (**108**) from *N. propolis*, mimosifolenone (**109**) from *Aeschynomene mimosifolia*, (*R*)-3,4-dimethoxydalbergiquinol (**110**) from *M. kuhlmannii*, latifolin (**111**) from *Belamcanda chinensis*, (*R*)-5-*O*-methyllatifolin (**112**) from various species of *Dalbergia*, 5-((1*R*)-1-(2-(acetyloxy)phenyl)-2-propen-1-yl)-2,4-dimethoxy phenol-1-acetate (**113**) from *D. latifolia*, 3-((1*R*)-1-(2,4,5-trimethoxyphenyl)-2-propen-1-yl)-phenol (**114**) from *D. odorifera*, pterolinus F (**115**) from *P. santalinus*, kuhlmanniquinol (**116**) from *M. kuhlmannii*, latinone (**117**) from *D. sissoo*, and some diaryl ketone like melanoxoin (**118**) from *D. melanoxylon* and *P. santalinus* and cearoin (**119**) from *D. coromandeliana* and *D. odorifera* as well as some chromene derivative, such as dalbergichromene (**120**) from *D. sissoo* and Kuhlmannene (**121**) from *M. kuhlmannii* have been isolated (Mukerjee et al. 1971a, b; Donnelly et al. 1975; Ollis et al. 1978; Donnelly et al. 1981; Fullas et al. 1996; Chan et al. 1997; Anjaneyulu et al. 2005; Awale et al. 2005; Sekine et al. 2009a; Wu et al. 2011; Edayadulla et al. 2012; Lee et al. 2013; Kumar et al. 2014; Peng et al. 2015).

3.3 Biological Activities of Neoflavonoids

3.3.1 Cytotoxic Activities

Wu et al. (2011) evaluated the cytotoxic action of sequestered neoflavonoids and found that the compound **84** possessed a significant cytotoxic activity against MDA-MB-231 (IC_{50} 3.34 μg/ml) and Hep3B (IC_{50} 2.39 μg/ml) cancer cell lines, whereas **89** exhibited a selective cytotoxicity against lung cancer cell line, A549 (IC_{50} = 3.97 μg/ml). Additionally, **118** displayed the uppermost cell toxicity effect against the cell line Ca9–22 (IC_{50} 0.46 μg/ml) cell line. On the other hand, compounds **122** and **3**, having lactone ring C, which is generally made by the cyclization of -C7-C8-C9-O-moiety on ring B were found not effective against the aforementioned cell lines. It means that the presence of heterocyclic ring C would have eliminated their cell toxicity effects. Melanoxoin, having carbonyl link between phenyl A and B rings instead of allyl group (CH-CH=CH_2), unveiled an effective antiproliferative activity against the oral squamous cell carcinoma cell line, Ca9–22. Thus, melanoxoin may be used as a potential anticancer drug (Wu et al. 2011). The compound **100** exhibited moderate cytotoxicity against colon 26-L5 (IC_{50} 60.3 μg/ml),

B16-BL6 (IC_{50} = 50.8 μg/ml), A549 (IC_{50} = 73.7 μg/ml), and HeLa (IC_{50} = 60.1 μg/ml) cell lines; however it is inactive against chronic lymphocytic leukemia (CLL) and colon cancer (HT-1080) cells. Doxorubicin and 5-fluorouracil were used as positive controls, and both compounds were found to exhibit cell toxicity against all the cells used in their investigations (Li et al. 2008).

In H1299 (human non-small cell lung cancer) cells, the compound **118** showed significant cytotoxic effects. The results also indicated that **118** regulated the cell cycles in H1299 cancer cells. The compound enhanced apoptosis in tumor cells due to the arrest of G2/M stage in cell cycle. Moreover, **118** induced substantial DNA damage and increased MET (proto-oncogene) expression in NSCLC (non-small cell lung cancer) cells. In addition, it was observed to hinder hepatocyte growth factor (HGF)-prompted cell migration as evidenced by the wound healing and transwell migration assays. Based on these results, **118** may be considered as a prospective anticancer agent (Peng et al. 2015). The compound **119** was tested for anticancer potential against the human neuroblastoma (SH-SY5Y) cells at different concentration with the help of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. It showed the elevation in the phosphorylation of the extracellular signal-regulated kinase (ERK), positive alteration of cellular protein LC3B-I to LC3B-II, reduction in the expression levels of Bcl2 gene, stimulation of caspase 3 enzyme, and cleaving of poly(ADP-ribose) polymerase (PARP) enzymes. These signaling enhanced the stimulation of autophagy and apoptosis in cancerous cells. The compound **119** also increased the generation of reactive oxygen species (ROS) and nitric oxide (NO) as determined by 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) fluorescence and Griess biological assays, respectively (Bastola et al. 2017).

3.3.2 Cardiovascular Activities

The nitric oxide free radical ($NO^{\cdot}$), created by NO synthase (NOS) function to protect host via injuring pathogenic genome (DNA) and possesses manifold biological functions. Also, it acts as controlling molecule to maintain homeostasis. Nevertheless, disproportionate creation of NO causes harmful effects in several organ systems, such as tissue damage and septic shock. Thus, inhibiting NO production in the body can be an effective healing approach for treating many diseases persuaded by pathological situations of ROS. The compound **90** inhibited the production of NO (IC_{50} values of 3.19 μM). It was having higher activity when compared to *N*G-monomethyl-L-arginine (a positive control), which showed IC_{50} value of 32.0 μM. Likewise, the compound **85** showed a stronger activity of NO inhibition (IC_{50} = 11.5 μM/ml) when compared to compounds **81** and **76**; both showed IC_{50} as 38.8 and 39.7 μM /ml, respectively. This indicated that the existence of an hydroxyl group at 4′-position can increase the bioactivity, and the occurrence or lack of a methoxyl group at 4′-position has no significant effect on NO production suppressive action of dalbergiones (Shrestha et al. 2008). Compounds **2** and **4** showed

strong inhibitory effects in the range of IC_{50} 35.1 to 72.0 μM/ml, whereas **111**, **114**, **112**, and **107** were found to inhibit NO production moderately with IC_{50} 70.3 to 74.0 μM/ml (Lee et al. 2013). Moreover, pretreatment with compound **111** inhibited the increase of changes in ECG (ST segment) levels in rats with myocardial ischemia induced by isoproterenol (ISO). Further, it considerably reduced the ISO-induced raise of serum amounts of the cardiac markers, such as cardiac troponin I (cTnI), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). This compound also maintained the myocardium membrane integrity through reducing cell inflammation and lowering down the level of interstitial edema. Additionally, **111** pretreatment facilitated the expression of nuclear and cytoplasmic nuclear factor erythroid 2-related factor 2 (Nrf2), NAD (P)H:quinone oxidoreductase-1 (NQO1), and heme oxygenase-1 (HO-1) in myocardial tissue in relation to ISO induction (Chen et al. 2016).

3.3.3 Antidiabetic Activity

Type 2 diabetes mellitus (T2DM) is one among several metabolic syndromes and is described by an increase of blood glucose levels in the circumstance of insulin deficiency and/or insulin resistance (Karalliedde and Gnudi 2014). The incidence of diabetes is growing rapidly globally, and it is anticipated that patients with T2DM will escalate to more than 0.629 billion by 2045 (IDF Diabetes Atlas 2017). The insulin resistance can be described by the incapability of cells to react adequately to usual levels of insulin. It is one the foremost pathophysiological defects in T2DM. Insulin resistance is found usually within liver, muscle, and fat tissues and leads to alter the total body glucose homeostasis. The skeletal muscles contribute to a higher postprandial glucose disposal and account for over 80% of insulin-reliant glucose disposal in humans (DeFronzo and Tripathy 2009). The uptake of glucose is the rate-determining stage in glucose consumption in diabetic and nondiabetic skeletal muscles (Perriott et al. 2001). In a study, coutareagenin (**12**) from *Hintonia latiflora* plant has been reported for antidiabetic effect on streptozotocin-induced diabetes in rats. Upon administering 100 or 200 mg/kg of *H. latiflora* extract, having coutareagenin as major component in both male and female rats, the blood sugar level was found to reduce at the same rate with nearly 42% (Korec et al. 2000). Therefore, the development of plant-derived α-glucosidase inhibitors is very critical in order to have high effectivity with low toxicity during the treatment of diabetes.

3.3.4 Antioxidant Activity

Glutamate excitotoxicity is involved in contributing to degenerative nerve diseases of the central nervous system (CNS), such as ischemia and epilepsy. These conditions may be connected to the receptor-instigated excitotoxicity or non-receptor-arbitrated ROS stress manifestation (Schubert and Piasecki 2001). Excitotoxicity means a disproportionate stimulation of amino acid receptors of neurons, and

glutamate-prompted excitotoxicity arbitrates the death of neurons in several disorders (Kritis et al. 2015). The HT22 (immortalized mouse hippocampal) cells phenotypically look like neuronal precursor cells; however they are deficient with active ionotropic glutamate receptors. The discounting of excitotoxicity causes glutamate-triggered cell death. Hence, HT22 cell lines are a suitable model to study about the oxidative glutamate cell toxicity. In a study, the compounds **80**, **111**, **112**, **101**, and **107** exhibited protective effects with EC_{50} values of 8.54, 5.82, 25.79, 6.54, and 8.14, respectively, against oxidative cellular injuries (An et al. 2008).

3.3.5 Antiplasmodial Activity

The compound **80** displayed strong anti-malarial activities with IC_{50} value of 5.8 μg/ml and inhibited the growth of *Plasmodium falciparum* strain FcB1, which is resistant to chloroquine (Beldjoudi et al. 2003). Likewise, the compound **34** also exhibited the potent anti-malarial action with IC_{50} value of 3.6 μg/ml against the *P. falciparum* strain poW, which is sensitive to chloroquine. Similarly, it showed IC_{50} value of 1.6 μg/ml against the *P. falciparum* strain Dd2, resistant to chloroquine (Kohler et al. 2001).

3.3.6 Anti-Allergic and Anti-Inflammatory Activities

Neutrophils (polymorphonuclear leukocytes) are the first line of host defense mechanisms against various pathogens. These cells play an important role as mediators in inflammation-induced injury conditions. Neutrophils, when activated, consume oxygen and form high levels of ROS and elastase enzymes. This activation of uncontrolled neutrophils gives rise to injurious effects and causes pathogenesis of many diseases, such as glomerulonephritis, emphysema, endothelial cell (EC) injury, rheumatoid arthritis, and tissue damage in ischemia-reperfusion states (Silvestre-Roig et al. 2016). Chan et al. (1998) isolated many flavonoids and neoflavonoids and then found that two neoflavonoids, namely, **81** and **119**, exhibit a significant inhibition of the histamine and β-glucuronidase release from mast cells of an experimental animal as observed in the superoxide formation and neutrophil degranulation experiments. The IC_{50} value of **81** for β-glucuronidase inhibition was recorded to be 17.6 μM, while for histamine, it was 17.9 μM. Likewise, the compound **119** showed IC_{50} value of 20 μM for β-glucuronidase and 16.3 μM for histamine release (Chan et al. 1998). In another experiment, the compounds, namely, melanettin, 30-hydroxy melanettin, latifolin, 5-O-methyllatifolin, 2,4,5-trimethoxy-3′-hydroxy-dalbergiquinol, and 4,5-dimethoxy-2-hydroxy dalbergiquinol, were isolated from *D. odorifera* and were assessed for their suppressive activity on NO generation in LPS (lipopolysaccharides)-encouraged RAW 264.7 cells (Lee et al. 2013). Among these compounds, only melanettin and 30-hydroxymelanettin displayed suppressive effects with IC_{50} values that ranged between 35 and 72 μM. Whereas, additional compounds displayed noteworthy suppressive effect on the generation of NO in LPS-prompted J774.1 cells. In

addition, Lee et al. (2013) investigated the anti-inflammatory property of latifolin. They found that it activated the Nrf2, which lead to the expression of HO-1. Subsequently, latifolin prevented the expression of iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) and decreased the expression of the pro-inflammatory mediators and cytokines, NO, prostaglandin E2 (PGE2), tumor necrosis factor (TNF)-α, as well as interleukin (IL-1β) in LPS-induced primary murine peritoneal macrophages. This experiment recognized the significant therapeutic properties of latifolin as a future medication for several inflammatory diseases (Lee et al. 2014). From *N. propolis*, three neoflavonoids, namely, 4-methoxydalbergione, dihydroxy-4-methoxydalbergione, and cearoin, were obtained. These compounds were shown to significantly prevent the stimulated mRNA expression of inflammatory genes, such as TNFα, IL-6, and IL-13 in BMMC (bone marrow-derived mast) cells. Thus, these compounds could promote healthiness and assist in preventing many inflammatory diseases (Tago et al. 2015).

3.3.7 Anti-Melanogenic Activity

In normal conditions, melanin protects our skin from the ultraviolet ray-induced injury. When melanin abruptly increases, it causes skin problems including sites of actinic damage and melasma spots. To overcome such types of problems, we need some alternative therapies, which can prevent the abnormal expression of melanin in the skin without causing any side effect (Kim et al. 2008). In search of these types of agents, two neoflavonoids, namely, 5,7-dihydroxy-4-phenylcoumarin and 7-Hydroxy-4-phenylcoumarin, were isolated and identified with potent anti-melanogenic activity as observed using the zebrafish experimental model. Both compounds 5,7-dihydroxy-4-phenylcoumarin and 7-hydroxy-4-phenylcoumarin inhibited melanin pigment at 5 mg/ml concentration; however, they failed to influence on the embryo development at the concentration of 50 mg/ml and 25 mg/ml, respectively (Veselinović et al. 2017).

3.3.8 Antimicrobial Activity

Cearoin (**120**) showed a significant antibacterial property against *Pseudomonas aeruginosa* and *Salmonella typhi* with the inhibitory concentrations of 15 and 18 mm, respectively (Shaheen et al. 2006). MAB 3 (**38**) displayed a potent activity (MIC = 32 mg/l) against *Escherichia coli* AG100A (Kuete et al. 2011). Yasunaka et al. (2005) sequestered mammea A/BA and mammea An/AA from the plant *C. brasiliense* and *Mammea americana*, respectively. Both these compounds exhibited higher inhibition activity against *Staphylococcus aureus* comparable to crude extracts (Yasunaka et al. 2005). (*R*)-4-Methoxydalbergione (**80**), isolated from the *D. nigra*, suppressed the growth of *Candida albicans* and *Bacillus anthracis* at lower concentration levels (20 to 100 μg/ml) (Jurd et al. 1971), while latifolin isolated from *D. latifolia* showed both antifungal and antitermite activities (Sekine

et al. 2009b). Interestingly, the mortality rate of termite was recorded to be only 26.7% after 14 days of treatment. Latifolin showed a significant percentage of inhibition of fungi, *Trametes versicolor* (79.1%) and *Fomitopsis palustris* (37.5%). However the fungal species *Rhizopus oryzae* and *Cladosporium cladosporioides* were not significantly affected by latifolin.

3.3.9 Antileishmanial Activity

C. brasiliense Cambess, belonging to Clusiaceae family has been investigated for its effect on leishmania parasites (Brenzan et al. 2007). They identified and evaluated antileishmanial effect of coumarin-type mammea compounds sequestered from the dichloromethane extracts of this plant leaves. The study results showed that (−) mammea A/BB exhibited a noteworthy action against amastigote and promastigote forms of *Leishmania amazonensis* with IC_{50} value of 0.88 and 3.0 μg/ml concentration, respectively. Besides it, coumarin (−) mammea A/BB demonstrated no cell toxicity against J774G8 macrophages cultured in vitro even at higher doses that suppressed promastigote forms.

3.3.10 Urease Inhibitor Activity

Urease is an enzyme, which finally involves in the hydrolysis of urea to form CO_2 and NH_3. (*R*)-Dalbergiphenol (**101**) with a single hydroxyl and 2 methoxy moieties exhibited superior suppressive activity with an IC_{50} value of 25.6 μM, while (*R*)-4-methoxydalbergione (**80**) without free hydroxyl group was reported with a lesser inhibitory activity with an IC_{50} value of 59.7 μM. This increased inhibitory activity due to a number of hydroxyl groups might be apparent because of the chelation of these phenolic moieties with Ni (nickel) in urease enzyme's active site. As a consequence, an enhanced inhibitory activity of the compounds was observed (Khan et al. 2006). Moreover, urease also takes part in the development of peptic ulcers and cancer because of the significant growth of *Helicobacter pylori* in the acidic condition of the gut. It is accounted for ureases, involved in causing urolithiasis and infection by *Proteus mirabilis* and *Yersinia enterocolitica*. The plant *Viola betonicifolia*-derived compound, 3-methoxydalbergione, was tested for the in vitro urease inhibition activity. This compound significantly proved as a remarkable inhibitor of urease enzyme with an IC_{50} value of 169 ± 2 μM (Muhammad et al. 2014).

3.3.11 Anti-Osteoporosis Activity

The severity of osteoporosis is ascertained by bone mineral density measurements (Feng and McDonald 2011). The loss in bone mass arises when osteoblasts fail to properly refill the cavity developed during resorption and the frequency of

osteoclast resorbing bone is greater than osteoblast depositing bone (Oursler et al. 1993). Bone resorption in females takes place in postmenopausal osteoporosis owing to increased activity and quantity of osteoclasts, contributing in the depletion of estrogen. Estrogen functions on osteoclasts as well as osteoblasts to suppress bone deterioration at all phases of life (Faienza et al. 2013). Compounds **6**, **7**, **101**, **118**, and **121** showed a significant osteogenic activity with an EC_{50} value of 1.015 nM, 91 μM, 98 nM, 116 μM, and 1.7 μM, respectively (Kumar et al. 2014). In another study, the effect of dalbergiphenol was studied on bone loss in ovariectomized (OVx) mice. Results of this experiment showed that dalbergiphenol prevented the bone loss either by increasing osteoblast activities or by decreasing osteoclast activities in OVx mice. On the basis of gene expression study, it is reported that dalbergiphenol increased mRNA expression of osterix, runt-related transcription factor 2, and collagen type 1 and reduced mRNA expression of tartrate-resistant acid phosphatase and osteoprotegerin-to-receptor nuclear factor-kB ligand ratio in the femur of ovariectomized mice (Gautam et al. 2015).

3.4 Conclusions and Future Prospects

In this chapter, different structures of neoflavonoids along with various pharmacological properties are highlighted. Calophyllolide was the first neoflavone isolated from the seeds of *C. inophyllum* in 1951. Neoflavonoids are mainly found in plant species, belonging to *Dalbergia* and *Calophyllum* genus. They are abundantly documented from the plant species of *D. odorifera*, *D. sissoo*, *D. nigra*, *D. latifolia*, *C. inophylloide*, *C. verticillatum*, *C. lankaensis*, and *C. thwaitesii*. In order to discover novel neoflavonoids, a large number of species of both the genus are still waiting for being surveyed and observed for their chemodiverse compounds using intense chemoprofiling research works. It is well known that medicinal plants and their phytochemicals are the current therapeutic targets for drug discovery and development. In this viewpoint, neoflavonoids have extensively been evaluated as therapeutic agents in the treatment of many diseases. Among them, dalbergin, dalbergione, methoxydalbergione, dalbergiphenol, cearoin, and latifolin are currently under extensive research studies, because of their wide-ranging pharmacological properties. Latifolin has notably been recently shown to protect the pituitrin or isoproterenol-induced myocardial injury through inhibiting oxidative stresses by activating Nrf2 signaling pathway. Dalbergiphenol can effectively prevent bone loss and help in overcoming the severity of osteoporosis. Dalbergin and dalsissooal were shown with potent osteogenic activities at very low concentrations of 100 pM and 1 pM, respectively. According to them, if oral supplementation having dalsissooal of *D. sissoo* is given, then it can improve bone structure by forming new bone cells and reduce bone turnover rate. Latifolin inhibits the protein and mRNA expression of inducible NO synthase and COX-2 and reduces NO, PE2, TNF-α, and IL-1β production in primary murine peritoneal macrophages. This chapter is a collection of several neoflavonoids that are being isolated and evaluated for their biological activities. As the literature survey confirmed their potential pharmacological properties,

neoflavonoids occurring in different plants may possibly be an attention-grabbing choice of molecules for herbal drug discovery and development. In this direction, the following points should be considered in the future to get desirable neoflavonoid compounds from plant sources: (1) different cultivars of *Dalbergia* species should be taken into account for future examinations, which may provide novel neoflavonoids in higher levels, and (2) the environmental factors, such as mineral and nutrients in soils, also affect the quality and quantity of phytocompounds, and therefore the geographic areas should also be analyzed and compared from where the material plant is to be taken. Further, the targeted neoflavonoids should be used in biomedical and pharmaceutical studies, involving in vitro, in vivo, and clinical trial steps to assess their remarkable pharmacological activities.

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4 Polyphenols' Role in Autoimmune and Chronic Inflammatory Diseases and the Advent of Computer-Driven Plant Therapies

Glaucia C. Pereira

Contents

Abstract

Oxidative stress is caused by an imbalance between reactive oxygen species (ROS) production and their removal via protective mechanisms. This severely contributes to the progression of inflammatory diseases. However, it is resident inflammation that leads to chronic states. To keep homeostasis, pro and anti-inflammatory cytokines modulate signalling cascades to defeat tissue impairment and infection. The underlying processes may cause chronic inflammation, if it remains continuously. Myriad of transcription factors are activated via oxidative stress, triggering protein expression involved in inflammatory pathways. Inflammatory signalling cascades are additionally close related to the establishment of side-conditions (e.g., myocardial infarct, stroke, kidney disease, atherosclerosis, diverse forms of cancer including glioblastoma multiform, and countless age-related ocular illnesses as cataract, glaucoma, diabetic retinopathy, and macular degeneration). To mention one example, atherosclerotic patients suffer from long lasting cholesterol-rich oxidative plaques deposition. To date,

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several inflammatory biomarkers have been examined, to evidence molecular activity to be targeted in therapeutics—e.g. interleukin families, nuclear factor-kappa B (NfkB), and signal transducer and activator of transcription 3 (STAT3). Polyphenols are anti-oxidative-rich compounds easily introduced in humans' daily diet, without major investments (polyphenols are found in for instance tea, whole grain wheat, rye, broccoli, asparagus, carrots, coffee, cocoa, olive oil, and red wine). Polyphenols' antioxidant capacity is demonstrated via their ability to modulate signalling pathways responsible for removing free oxygen and nitrogen species, mitigating the enzymatic promotion of pro-inflammatory activity—e.g. modulating the expression of cyclooxygenase (COX), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS). Additionally, these hydroxyl-bonded groups of chemical compounds have demonstrated high potential as vasodilators, which can contribute to reducing risk of cardiovascular diseases. Regardless the fact knowledge on polyphenols properties is well-stablished, in the current literature, these properties have been poorly exploited, lacking accurate assessment of polyphenol metabolites as biomarkers (e.g., kaempferol, myricetin, quercetin, naringenin, hesperetin, epicatechin, gallocatechin, epigallocatechin, epicatechingallate, epigallocatechingallate, and procyanidin), among high risk patients, potentially using low risk groups as a control. Additionally, chemical instability of certain herbal medicines combined with needs for improving delivery systems to optimise effectiveness may challenge the success of phytotherapies. Therefore, it urges designing new formulations (e.g., modifying pharmacokinetic profiles) and novel delivery systems that optimise administration routes, e.g. exploiting the low cytotoxicity and biocompatibility of nanoparticles as polysaccharides. To mention one example, hypericin has been tested in photodynamic therapies (precision medicine), due to its photocytotoxic effects inducing apoptosis. Ultimately, computer-assisted approaches may add value by reducing efforts in-production, providing robust tools for pre-laboratorial design and testing. Therefore, in this Chapter, major inflammatory biomarkers will be discussed, limitations and potential of herbal therapies will be presented, and novel therapies debated (including the ones benefitting from computer-aided systems).

Keywords

Polyphenols · Autoimmunity · Autoimmune type of atherosclerosis · Epigenetics · Omics · Oxidative stress · ROS · RNS · Inflammatory biomarkers · Chronic inflammation

4.1 Introduction

According to the World Health Organization (WHO 2018), inflammation is severely influencing mortality rate, worldwide. This is because, ischaemic heart disease, stroke, chronic obstructive lung disease, and varied chronic affections are among the major causes of death, globaly. Therefore, ensuring appropriate biomarkers assessment, optimised phenotypic drug tests and efficient drug delivery systems for chronic inflammatory pathologies should be a priority for the wide scientific community. Greater efforts are needed to ensure effective drug discovery and repurposing. The goal would be to target medicinal compounds aiming at optimising therapeutic benefits, reducing side effects. Plant metabolites are proven strong candidates for improving clinics. Hence, debate on the role of polyphenols mitigating autoimmune and inflammatory signalling cascades is found in a considerable volume of work, in the current literature (Dangles 2012; Chuang et al. 2013; Chew et al. 2019).

Immunotherapies are strongly contributing to leverage the burden of immune impairment, by assessing diseased immune signalling cascades via the induction, enhancement, and suppression of immune responses; to design the most appropriate therapeutic approach (Wraith 2017). Continued efforts are needed to target immune responses, correctly identifying autoimmune trends in disease initiation and progression. Indeed, numerous chronic affections may potentially be classified as autoimmune illnesses. However, disease profiling is challenging. One example of chronic inflammatory pathology with a high potential to be classified under the perspective of autoimmunity is atherosclerosis. Therefore, we here discuss fundamental trends in atherosclerosis initiation and progression, indicating autoimmune profiling. Atherosclerosis commencement may be strongly related to autoimmunity-driven cell apoptosis, due to impaired redox leading to DNA damage, cell cycle arrest, and damage to the cell membrane. This information can be used to develop targeted interventions aimed at mitigating atheroma development. Therefore, this chapter invites the reader to immerse in debate on polyphenols-driven modulation of chronic inflammation and autoimmune signaling cascades; deliberating immune impairment and inflammatory biomarkers, and discussing the current literature on novel terapeutics. Ultimately, the benefits of computational automation and forecasting are debated, leveraging biomedicine (Pereira 2017c; Peng et al. 2019; Bezerra-Filho et al. 2019; Aulner et al. 2019; Wang et al. 2019a).

4.2 Major Biomarkers in the Progression of Chronic Inflammatory Diseases and Atherosclerosis under the Perspective of Autoimmunity

Biomarkers for chronic inflammation have been broadly identified in the current literature (Tedgui and Mallat 2006; McInnes and Schett 2007; Di Paolo and Shayakhmetov 2016; Wang et al. 2016, b; Pereira 2017a, b; Liu et al. 2017; Waters et al. 2019; Li et al. 2019; Murphy 2019; Du et al. 2019; Ayala et al. 2019; Sharma et al. 2019; Henrik Heiland et al. 2019; Furiati et al. 2019; Park et al. 2019;

Engelbertsen et al. 2019; Insuela et al. 2019; Hwang et al. 2019). They vary widely, from growth factors, enzymes, and lipoproteins to reactive oxygen species (ROS). However, special attention is given to families of cytokines (Vignali et al. 2008; Van Tits et al. 2011; Pereira et al. 2014; Di Paolo and Shayakhmetov 2016; Pereira 2017a; Henrik Heiland et al. 2019). It is widely accepted that chronic inflammation results from resident inflammatory signalling cascades, where medicinal compounds have little or no effect mitigating disease progression. To date, numerous trends have been discussed. Examples are (i) disparity between ROS production and their removal, increasing oxidative stress (Pereira et al. 2014; Pereira 2017a); (ii) either oxidative environments or multiple growth factors triggering activation of transcription factors (e.g. nuclear factor-κB (NF-κB)), which are directly associated with immune responses (Pereira et al. 2014; Pereira 2017a; Liu et al. 2017); (iii) gene expression triggering wide-ranging innate immune cell (e.g. monocytes) activity, which ultimately responds to pathogen invasion (Kamada et al. 2008; Mahabeleshwar et al. 2011; Sharma et al. 2019); and (iv) somatic hypermutation of B cells related to adaptive immune responses (Häusser-Kinzel and Weber 2019; Arneth 2019).

Chronic inflammation can promote the development of varied pathologies, resulting in a large and growing body of literature dedicated to the investigation of satellite trends. Indeed, the spectrum of satellite pathologies caused by resident inflammation goes from eye diseases like glaucoma (Bodh et al. 2011; Chen et al. 2018; Barış and Tezel 2019) and diabetic retinopathy (Wong et al. 2016; Sorrentino et al. 2018) to kidney disease (Mihai et al. 2018; Sirac et al. 2018; Gomez and Sequeira-Lopez 2018; Onal et al. 2019), among other affections. To mention a few works in the field, in a recent study by Riehle and Bauersachs (Riehle and Bauersachs 2019), inflammatory biomarkers were correlated with heart failure, with special attention given to cases where ejection fraction is preserved (HFpEF). Interestingly, the authors highlighted two opposing aspects of inflammatory mechanisms, in disease progression. First, the role of inflammation, as a promoter, in the pathogenesis of heart failure is investigated. Second, the activation of regenerative immune responses following acute myocardial injury was discussed, to elucidate therapeutic targets. In that study, the authors indicated a number of risk factors, in the progression of HFpEF—e.g. renal failure, arterial hypertension, chronic obstructive pulmonary disease (COPD), metabolic syndrome, diabetes mellitus, and iron deficiency. Systemic resident inflammation promoted by these comorbidities was correlated with increased production of mitochondrial ROS, raising peroxynitrite ($ONOO^-$) levels, and reduction in nitric oxide (NO) levels in endothelial cells. Altogether, this affects cardiomyocyte soluble guanylate cyclase (sGC)/guanosine monophosphate (cGMP)/protein kinase G (PKG) signalling cascades, leading to left-ventricular (LV) dysfunction. Moreover, a positive correlation was found between increasing myocardial inflammation and cardiac fibrosis, because fibroblasts differentiation into myofibroblasts is triggered by growth factor beta (TGF β) expression in monocytes. These trends are crucial for understanding HFpEF progression, because this pathology results from LV relaxation, due to changes in both extracellular matrix composition and guanosine monophosphate (cGMP)/protein kinase G (PKG) signalling.

As indicated in the former paragraphs, discussion on the adverse effects of resident inflammation are in Riehle and Bauersachs (2019) followed by the appreciation of the role of inflammatory signalling in restoring impaired tissue, succeeding acute myocardial injuries. In that study, the authors focused on mice models of cardiovascular inflammation, in acute myocardial events, reporting on the role of macrophages, monocytes and myofibroblasts in reparative neovascularisation and fibrosis of the infarct zone. Pro-inflammatory cytokines—e.g. IL-1β and tumour necrosis factor alpha (TNFα); combined with anti-inflammatory and pro-fibrotic cytokines—e.g. IL-10 and TGFβ; modulate monocytes migration to the injury site, to degrade damaged tissue. Subsequently, upregulation of smooth muscle α-actin (smα) in response to profibrotic agents (e.g., TGFβ) mediates fibroblasts differentiation into myofibroblasts, to restore the infarcted area (Ong et al. 2018). Therefore, the indicated inflammatory process is fundamental for cardiac tissue repair. However, imbalance in the expression of pro and anti-inflammatory cytokines, following myocardial infarct, may lead to resident inflammation (Van Linthout and Tschöpe 2017; López et al. 2019). Thus, post-acute myocardial injury therapies based on the intercellular modulation of inflammatory phenotypes, in response to secretion of corticosteroids—e.g. via glucocorticoid receptor (GR) expression; would circumvent resident inflammation, preserving contractile function and regulating tissue repair (Suthahar et al. 2017; Galuppo et al. 2017).

In recent days, major studies on resident inflammation-driven ischemic stroke have emerged (Acosta et al. 2019; Hong et al. 2019a). Some authors, however, debate inflammation as a consequence of ischemic stroke, leading to diverse brain diseases, such as cognitive and motor impairment (Zhang et al. 2019a). We wish to stress findings presented by Esenwa and Elkind (Esenwa and Elkind 2016), in 2016, because systemic inflammation causing stroke is discussed by the authors under the perspective of the design of immune system's modulators, which would ultimately knockdown signalling pathways that trigger ischaemia. In this study by Esenwa and Elkind (2016), chronic infections caused by pathogens such as *Chlamydia pneumoniae*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, herpes simplex virus (HSV) 1 and 2, human cytomegalovirus (CMV), influenza virus, and Epstein-Barr virus were linked to high risk of ischaemic stroke, because of associated resident low-grade inflammation. This would drive our attention to immunisation, as a preventive measure, mitigating both resident inflammation and, ultimately, ischemic stroke. Interestingly, in that work, the authors indicated that correlations between inflammation driven chronic infection and cardiovascular diseases (CVDs) were not clear, for lacking conclusive evidences from antibiotic treatment trials. The authors stress the role of an immune response protein, the C-reactive protein (CRP), whose expression in the liver is modulated by IL-6. This is because CRP was found to induce both atherogenic and thrombogenic signalling. Additionally, the combined effect of inflammatory biomarkers as lipoprotein-associated phospholipase A2 (Lp-PLA2), fibrinogen and tumour necrosis factor (TNF) was discussed. Lp-PLA2 driven hydrolyses of low-density lipoproteins (LDLs) was positively correlated with chronic inflammation and atherosclerotic plaques formation, due to the formation of oxidised phospholipids. However,

the most interesting observation made by the authors was the role of fibrinogen, IL-6 and TNF in the recurrence of stroke.

Challenging aspects of chronic inflammation derived diseases' progression may arise from autoimmunity. Thus, we wish to examine autoimmunity related atheroma development, because atherosclerosis is a burden of mortality, in modern societies, having its roots currently not fully identified. Before proceeding to examine autoimmune responses associated to lipid plaque deposition, it is important to understand the motivation for the underlying assumption, as atherosclerosis is not yet known to be an autoimmune pathology. In a work presented in 2013 (Pereira 2013), the author discussed varied aspects of atheroma progression and its inflammatory fingerprints, to both introduce a computational model of the disease and start investigating autoimmunity related pathological signalling cascades. Time passed and those investigations progressed (Pereira 2017a, b), resulting in a more elaborated hypothesis on an autoimmune type of atherosclerosis. Recently, other authors have shown interest in investigating autoimmunity-related atheroma formation (Matsuura et al. 2014; Sima et al. 2018; Sanjadi et al. 2018), corroborating the relevance of such hyphotesis in the assessment of atheroma initiation and progression.

When discussing autoimmunity, one needs to consider whether a legitimate inflammatory signalling cascade initiated the imbalanced immune response. That is, if immune mechanisms were legitimately activated by a pathogen or result from impaired physiology. One shall not argue on whether inflammatory biomarkers were observed nor if immunological impairment took place, because the answer for these questions are well-known by the scientific community and inflammatory diseases share similar fingerprints. Focus might be on immune signalling not triggered by inflammatory tissue impairment. In an interesting article by Abou-Raya et al. (ABOU-RAYA et al. 2007), similarities between rheumatoid arthritis (RA), periodontal disease (PD), coronary artery disease (CAD), and atherosclerosis were discussed. However, again, showing common inflammatory biomarkers in disease progression does not elucidate disease initiation. Therefore, genotypic similarities between an autoimmune pathology such as rheumatoid arthritis and atherosclerosis do not imply classifying atherosclerosis as an autoimmune disease. Indeed, while the incidence of coronary artery disease in patients suffering from autoimmune disorders is high, patients not suffering from autoimmune conditions are not protected against CAD. This implies autoimmunity seen as a risk factor not the ultimate causation. Both in this chapter and in past research, the author argues on whether there is enough evidences on atheroma formation being initiated without an obvious cause, investigating initial stages in disease progression, prior to and during ox-LDL formation and monocytes migration and differentiation into microphages.

Both focal high concentration of LDL and increasing cell interfacial gaps promote LDL migration into the endothelial wall. In atheroma formation, this initial step is followed by the production of ox-LDL, which triggers immune responses, to remove this metabolic waste from the artery wall. The author here proposes that this is not the point where key immune responses take place. Indeed, the author's hypotheses are on immune impairment related to both pathological production of reactive oxygen species (ROS) and abnormal cell apoptosis, along with ROS driven DNA

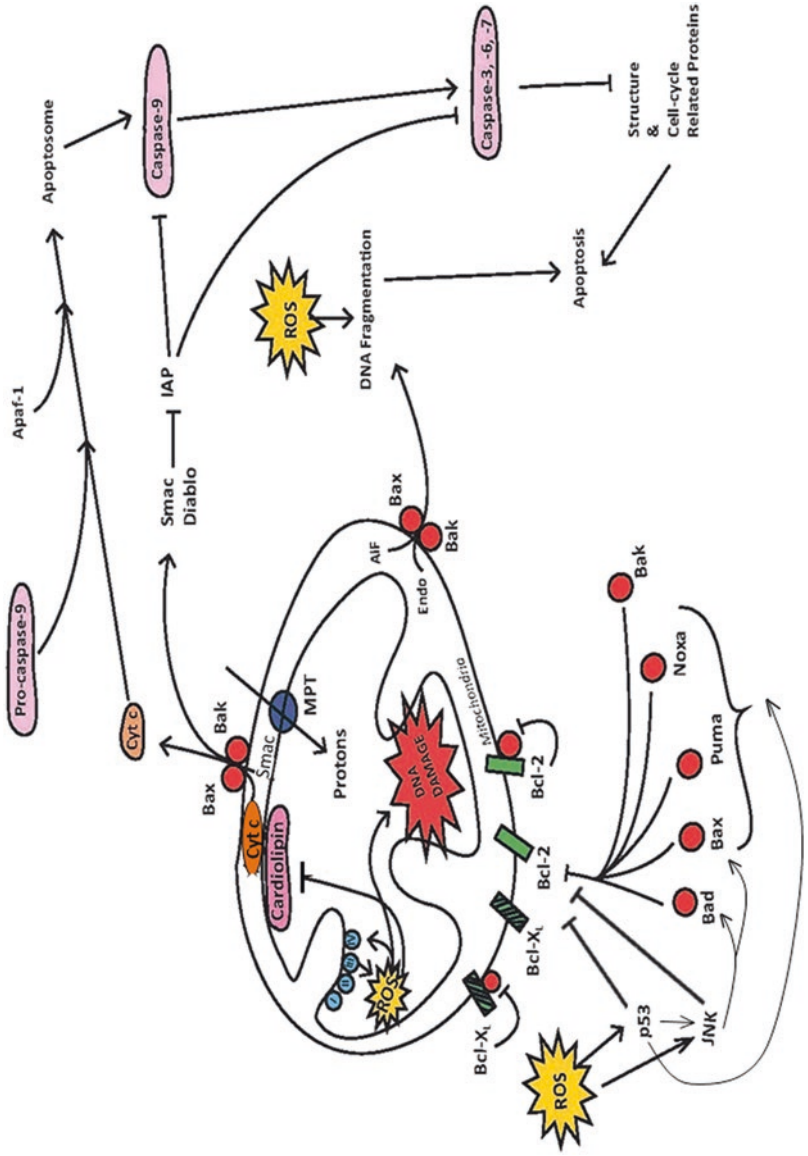

Fig. 4.1 Illustration of ROS generation and its effects — e.g. DNA damage, cell cycle arrest, alterations in apoptosis, and damage to the cell membrane [with copyright, Redza-Dutordoir and Averill-Bates 2016]

damage. This is because, it would indicate a process orchestrated by an abnormal concentration of ROS, in which one's immune system contributes towards degrading one's cells. Fig. 4.1 illustrates well the key elements that are indicated in this paragraph, though in a different context. Over the last 15 years, the author has investigated chronic inflammatory biomarkers related to satellite diseases, such as atherosclerosis, cancer and CVD. This drove attention to the role of oxidative stress and cellular dysfunction, in autoimmune pathologies as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren's syndrome (SS). Noticing that similarly to what is observed in initial stages of atheroma formation, a pathological oxidative stress profiling contributes to the pathogenesis of autoimmune diseases by increasing (amplifying) inflammatory responses, inducing apoptotic cell death, causing abnormal immunological activity (Bashir et al. 1993; Kumagai et al. 2003; Victor 2014; Ates et al. 2015; Harrison et al. 2019). The last is key in the characterisation of autoimmune diseases and are therefore the foundations of the evidences presented by the author, in this chapter.

While investigating trends associated with myriad other diseases and multiple aspects of atheroma development (Das 2004; Muñoz et al. 2010; Franks and Slansky 2012; Turner et al. 2013; Victor 2014; Panth et al. 2016; Straub and Schradin 2016; Pereira 2017a; Moris et al. 2017; Schlüter et al. 2017; Duan et al. 2019; Paone et al. 2019; Tsygan et al. 2019; Hong et al. 2019b), the research community has been reporting on several evidences that, collectively, naturally drive us towards concluding that there is an autoimmune-type of atherosclerosis. However, to the best of the author's knowledge, these evidences were not collated nor analysed in a combined manner, properly correlating relevant patterns, and responding to the right questions. This ultimately means investigating autoimmune mechanisms as an initiator in atherosclerotic plaque build-up. Hence, over the last years, the author has argued on the causes of increasing cellular apoptosis in atherosclerosis commencement, concentrating on the role of pathological ROS production. Additionally, although ox-LDL production in the inner arterial wall is a well-known mechanism intended to promote lipid removal from the artery, by the immune system, the author here highlights resident oxidative environments augmenting ox-LDL production, causing defective immune responses and ineffective phagocytosis. Therefore, the underlying argument in favour of atheroma formation and autoimmunity correlation is that insights were found on similar defective immune mechanisms present in both autoimmune profiling and atherosclerosis origination. Indeed, as above indicated, focal pathological ROS levels are proven to be related to oxidative DNA alterations (Bashir et al. 1993). Taken together, the former suggests that the author's hypotheses on that such alterations may be causing both changes in phagocytosis signalling (dysfunction) leading to plaque deposition and immune responses that promote cell apoptosis are well founded, with key elements related to immune responses to altered DNA, similarly to what is observed in pathogens' invasion. Again, focal abnormal cell apoptosis is among the major factors related to the initiation of atheroma development. Therefore, it is sensible driving research in that direction, aiming at targeting autoimmune therapies, to be repurposed for mitigating key trends in atheroma formation. Together with preventive measures, this might mitigate harmful levels of ROS production, in the human body.

4.3 Polyphenol-Driven Modulation of Signalling Pathways in both Chronic Inflammation and Autoimmune Pathologies

The speed at which polyphenols-based treatments are progressing is remarkable. This is due to polyphenols' important role preventing harmful levels of oxidative stress, mitigating chronic oxidative and inflammatory diseases progression. Phenols anti-inflammatory and anti-oxidative action is complex. However, one beneficial phenols-based signalling pathway relates to the production of quinones. Chromic acid triggered phenols oxidation results in the production of 1,4-benzoquinone (a dicarbonyl known as quinone). Phenols' oxidation products can be reduced to dihydroxybenzene and reverse reactions achieved via varied reducing agents—e.g. NaBH4. Dihydroxybenzene is an important anti-oxidative agent, because it contributes towards reducing reactive oxygen species and supports recovering antioxidant α–tocopherol chains (Dangles 2012).

Dihydroxybenzene can be correlated with numerous inflammatory oxidative signalling cascades, which are known to modulate progression of varied pathologies—e.g. autoimmune and neurodegenerative diseases, chronic arterial inflammation, cancer, and multiple metabolic disorders. To mention a few examples, hydroquinone-based proelectrophilic small molecules were found to activate transcription factor Nuclear Factor (erythroid-derived 2)-like 2 (Nrf2) and HSF-1 pathways (Satoh et al. 2015). Nrf2 is proven to modulate the expression of about 250 genes that encode a network of enzymes promoting antioxidant metabolism, intermediate metabolism of carbohydrates and lipids, regulate inflammatory signalling, and control both iron catabolism and protein degradation (Cuadrado et al. 2019). Additionally, Nrf2 was found to reduce the severity of autoimmune affections by inhibiting dendritic cells' function, because Nrf2 activation is thought to reduce the production of activated T helper cells (Th1/Th17) and T cell proliferation (Hammer et al. 2017). Ultimately, Nrf2 can be also intrinsically related to the action of transcription factors like the NFkB, modulating chronic inflammatory signalling cascades (Pereira 2017c).

Another significant aspect of polyphenol activity is their role in the upregulation of glutathione expression (Visioli et al. 2009; Ulewicz-Magulska and Wesolowski 2019). The modulation of glutathione levels in the human body has multiple applications, because this peptide can both reduce α-tocopherol radicals and contribute with glutaredoxins and glutathione S transferase (GST) signalling cascades, to clear the human body from reactive electrophilic molecules (Richter and Kietzmann 2016).

Reactive oxygen species are important for keeping homeostasis, if produced in well-controlled proportions, within the human body. In health condition, ROS contribute to gene expression and signal transduction and hold imperative role in increasing production of adenosine triphosphate (ATP), during exercise, via aerobic metabolism (Radak et al. 2005, 2013; He et al. 2016). However, continuous exposure to excessive levels of ROS can cause significant damage to cell DNA and RNA, proteins, and lipids. Interestingly, ROS metabolism-driven exercise is complex. Indeed, while well-designed physical activity induces adaptive responses to increasing ROS production, regulating redox systems, harmful increase in the

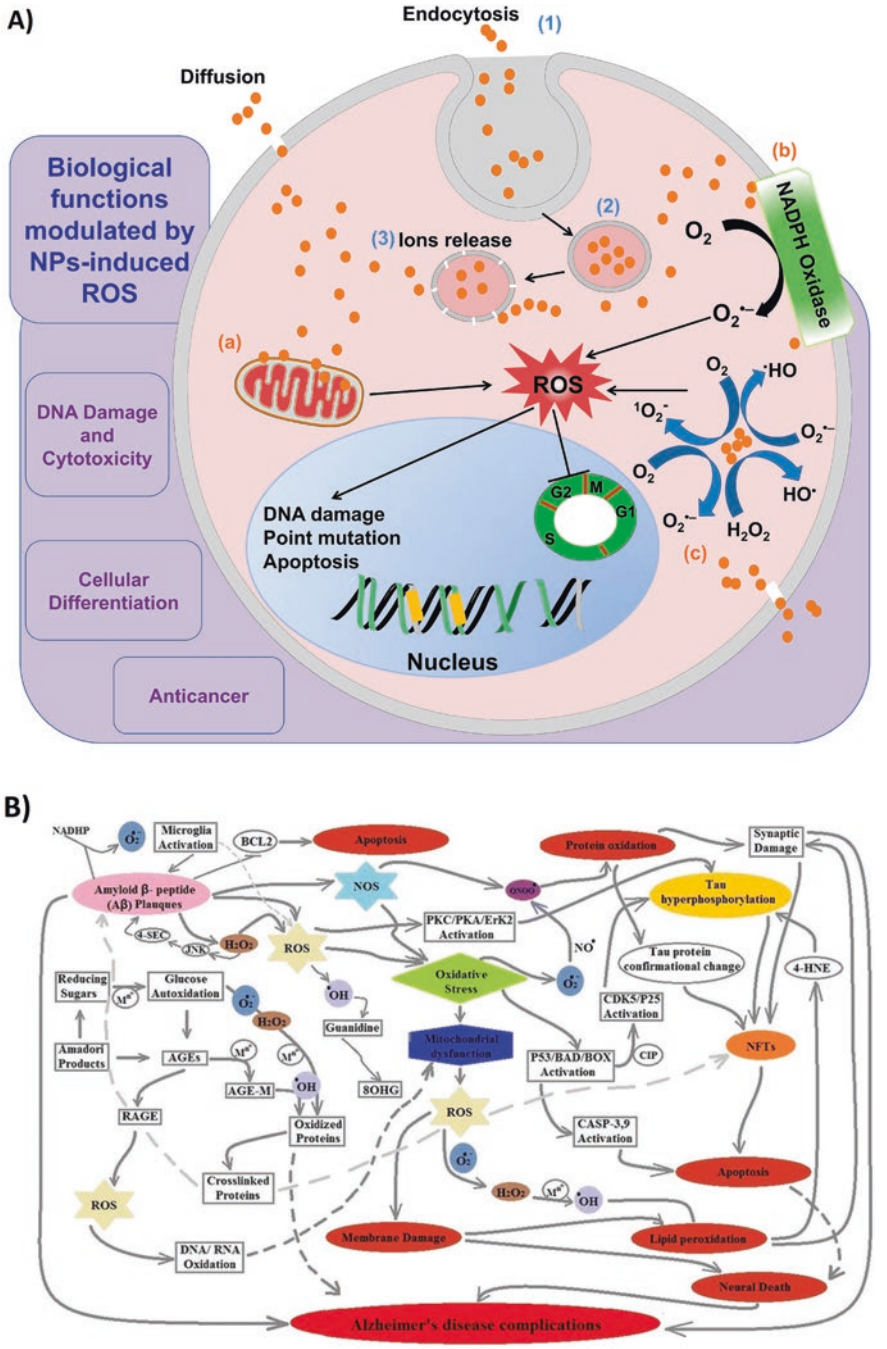

Fig. 4.2 Reactive oxygen species altering cellular functions: (**a**) beneficial usage of ROS in nanoparticle (NP)-based therapies [with copyright, Dayem et al. 2017] and (**b**) naturally occurring ROS negatively affecting redox [with copyright, Ahmad et al. 2017]

concentration of reactive oxygen species is thought to correlate with exhaustive muscle contraction (Zuo et al. 2015), which combined with impaired redox mechanisms can give raise to cellular damage.

The negative effects of overexposure to high concentrations of both ROS and reactive nitrogen species (RNS) are closely related to pre/neoplastic cell alterations, causing cellular apoptosis and necrosis. The last is currently being explored in novel cancer therapies (Fig. 4.2a), using nanotechnology, aiming at optimising precision medicine (Levy et al. 2019). However, in the context of naturally occurring ROS and RNS production, within the human body, redox imbalance is among the key promoters in signalling cascades triggering varied pathologies, including chronic inflammation and autoimmune diseases, which in Alzheimer's disease relates to Aβ plaques and hyper-phosphorylated tau neurofibrillary tangles (T-NFTs) promoting oxidative damage (Fig. 4.2b). Given the background, phenolic compounds can inhibit both ROS and RNS imbalances, preventing deterioration of cellular viability.

Polyphenols have also shown a great potential to treat neurodegenerative conditions. To exemplify, Alzheimer's disease is driven by Amyloid-β (Aβ) neurotoxicity induced by ROS formation, which causes neuronal cells' death due to glutathione deficiency (Cao et al. 2018). Additionally, varied neuronal impairment is caused by resident inflammation signalled via high concentration of interleukin families (e.g., IL6, IL1β, IL12, and IL18) and tumour necrosis factor alpha (TNF-α) (Wang et al. 2018). Polyphenols contribute to the control of Aβ-induced neurotoxicity and maintenance of synapses via cyclic adenosine monophosphate (cAMP) binding mechanisms (Ibrahim Fouad and Zaki Rizk 2019; Acquarone et al. 2019), additionally modulating important inflammatory biomarkers, as indicated in the above paragraphs. Inflammatory biomarkers including interleukin families are likewise imperative in the progression of multiple sclerosis (MS) (Kacperska et al. 2015; Göttle et al. 2019), while Ischaemic stroke was discussed in former sections, with a focus on chronic infections leading to inflammatory signalling, fibrinogen, along with fibrinogen, IL-6, and TNF cascades (Esenwa and Elkind 2016). Finally, NFkB modulates resident inflammation and cellular death, which are crucial measures in ischaemic stroke (Zhang et al. 2005). It is now understood that polyphenols play an important role in reduction of inflammatory impairment, contributing to the modulation of degenerative mechanisms that characterise MS progression (Katz Sand 2018; Herden and Weissert 2018). Additionally, similar phenols' properties corroborate with both lowering risk of stroke and reducing the severity of the resulting pathophysiology (Goszcz et al. 2017; Patnaik et al. 2019), by acting on multiple signalling cascades, which include fibrinogen, IL-6, NFkB, and TNF pathways. Lastly, research shows that quercetin modulates both calcium dysregulation driven by acid-sensing ion channel and lipid peroxidation in neural cells, attenuating the burden of ischaemic injury (Pandey et al. 2011). As a final point, genetic disorders as Parkinson's disease (PD) and Huntington's disease (HD) are complex. HD is an inherited neurodegenerative disorder caused by a mutation in the CAG trinucleotide repeat segment, within the HTT gene (Zeitler et al. 2019), while PD's characteristic cognitive deterioration is thought to be associated with mutations in SNCA, LRRK2,

PRKN, DJ1, PINK1, and ATP13A2; common variation in MAPT, LRRK2, and SNCA (Bekris et al. 2010); and several other pre-conditions. However, on top of genetic factors driving causality in both diseases, there are intrinsic inflammatory mechanisms (Thomas 2014; Nagamoto-Combs and Combs 2014; Thomas and Adams 2014), which are a potential target for phenol-based compounds. Neurodegenerative diseases tend to share similarities—e.g. mitochondrial dysfunction modulating pathogenesis of PD, AD, and MS (Wang et al. 2015; Sadeghian et al. 2016; Park et al. 2018; Thubron et al. 2019; Foti et al. 2019). Therefore, existing polyphenols drugs can be extremely beneficial. At scale, they can be repurposed, to shorten long-lasting Food and Drug Administration's (FDA) drug approval processes.

4.4 Challenges and Progress in Herbal Therapies

The past decade has seen the rapid development of plant extract-based drug discovery and its increasing benefits. In recent years, improved techniques to isolate medicinal compounds from *Rabdosia rubescens*' leaves were developed, giving raise to oridonin ($C_{20}H_{28}O_6$) compound, which exhibit important anti-inflammatory properties (He et al. 2018), modulating expression of multi-protein oligomers that alter pro-inflammatory cytokines signalling pathways. *Alisma canaliculatum* (AC) comprising of 10-O-methyl-alismoxide, 11-deoxyalisol C, 4-12-dihydroxyguaian-6,10-diene, alismol, alismoxide, alisol B, alisol B 23-acetate, alisol C 23-acetate, and alisolide; deriving from *Cheong-Sang-Bo-Ha-Tang* (CSBHT) is thought to downregulate NFkB and inducible nitric oxide synthase (iNOS), which results in anti-asthmatic effects related to the indicated anti-inflammatory properties (Shin et al. 2014). The value of CSBHT is vast. Indeed, in a study published in 2017 (Kim et al. 2017), Kim et al report on the effects of CSBHT derived formulas mitigating radiation-triggered lung inflammation.

Plant secondary metabolites were discussed as valuable natural compounds in anti-malarial therapies (Pereira et al. 2019). *Artemisia annua*, which is the target herb in that work, exhibits antioxidant and anti-inflammatory properties that are broadly discussed in the current literature (i) as regulators of immune mechanisms—e.g. via upregulation of CD8 + T lymphocytes and downregulation of B cell responses (Zhang et al. 2019b); and (ii) in plant morphology-based therapeutic assessment—e.g. by evaluating variations in compound's antioxidant activity, based on its sources, among the various structural parts of *Artemisia* specie (Lee et al. 2015). Both cyclooxygenase (COX) and lipoxygenase (LOX) are leading biomarkers in progression of numerous physiological imbalances. COX-1 and COX-2 are involved in the pathophysiology of osteoarthritis, atherosclerosis, and cancer (Koki et al. 2002). These enzymes are also associated with neuronal inflammatory injuries, causing cognitive deterioration, via ox-dopamine-triggered signalling cascades and prostaglandin E2 receptor EP2 subtype activity (Kang et al. 2017). Lipoxygenases (LOX) add to ROS activity, causing lipid oxidation, significantly contributing to increasing risk of chronic inflammatory diseases. LOX is linked to hydroperoxide production, in the human body, which in disease condition may lead to production

of volatile carbonyls (Zoia et al. 2011; Ratnasari et al. 2017). Plant compounds are efficient inhibitors in the pathological activity of both LOX and COX (Dohi et al. 1991; Peng et al. 2006; Sharma and Singh 2010; DUQUE et al. 2011; Czubinski et al. 2012; Owczarek and Lewandowska 2017; Ma et al. 2017).

Undoubtedly, herbal therapies are suitable alternatives for conventional treatments. To exemplify, in cancer research, combined with nano delivery systems, plant compounds can potentially eliminate the risk of cellular toxicity that is posed by chemotherapy (Squillaro et al. 2017, 2018a, b). In epithelial affections driven by ROS production and inflammation, the selective activation of Nrf2 by plant extracts such as curcumin and sulforaphane could prevent side effects caused by commonly used drugs—e.g. ketoconazole; which may increase oxidative stress damage (Gęgotek and Skrzydlewska 2015). Benefits of herbal therapies and variety in plant metabolites make plant extracts very attractive in biomedicine. Given the background, the usage of polyphenols with medicinal purposes is highly recommended, because these compounds can be easily added to the daily diet—e.g. tea, whole grain wheat, rye, broccoli, asparagus, carrots, coffee, cocoa, olive oil, and red wine are polyphenol-rich. However, besides existing efforts to map phenols to target phenotype (DUQUE et al. 2011; Kozłowska and Szostak-Węgierek 2018), further advancement is compromised by lacking accurate assessment of polyphenol metabolites as biomarkers, on the basis of both high risk patients and low risk groups as a control. Additionally, chemical instability of certain herbal and flaws in existing drug delivery modes are still challenging phytotherapeutics (Li et al. 2008; Yan et al. 2015; Idrees et al. (in press)). Ultimately, in Pereira et al. (2019), we discussed biotechnological approaches to improve extraction modes of *Artemisia annua*, resulting in higher yield, as low yield is a major flaw in artemisinin production. In general lines, it urges optimising herbal-driven drug discovery, by strengthening compound activity via advanced delivery modes—e.g. using nanoparticles as delivery systems; and increasing compound availability via enhanced extraction approaches.

4.5 Computer-Aided Approaches in Polyphenolic Therapies

Over the last decades, great amount of effort was devoted to target new metabolites in personalised medicine (Peng et al. 2019; Bezerra-Filho et al. 2019; Wang et al. 2019a), screening libraries were built to leverage genotype-phenotype matching in drug discovery and repurposing (Pereira 2017c; Aulner et al. 2019), and drug delivery was empowered by nanocomposites in precision medicine. Computer science is a strong ally of biotechnology, resulting in simple methodologies that give rise to sophisticated synthetic biology design. Artificial intelligence and parallelisation are optimising decision making, regarding both speed and volume of information analysed (Fig. 4.3). However, this is one single example of what computing can do for biomedicine.

The horizon of computing-based solutions seems limitless, from recombinant DNA technology leveraged by computer-aided genetic engineering design (Adleman 1994; Rothemund et al. 2004; Qian et al. 2011; Gould et al. 2014; Pereira 2017c), artificial intelligence applied to both smart construction of screening libraries and

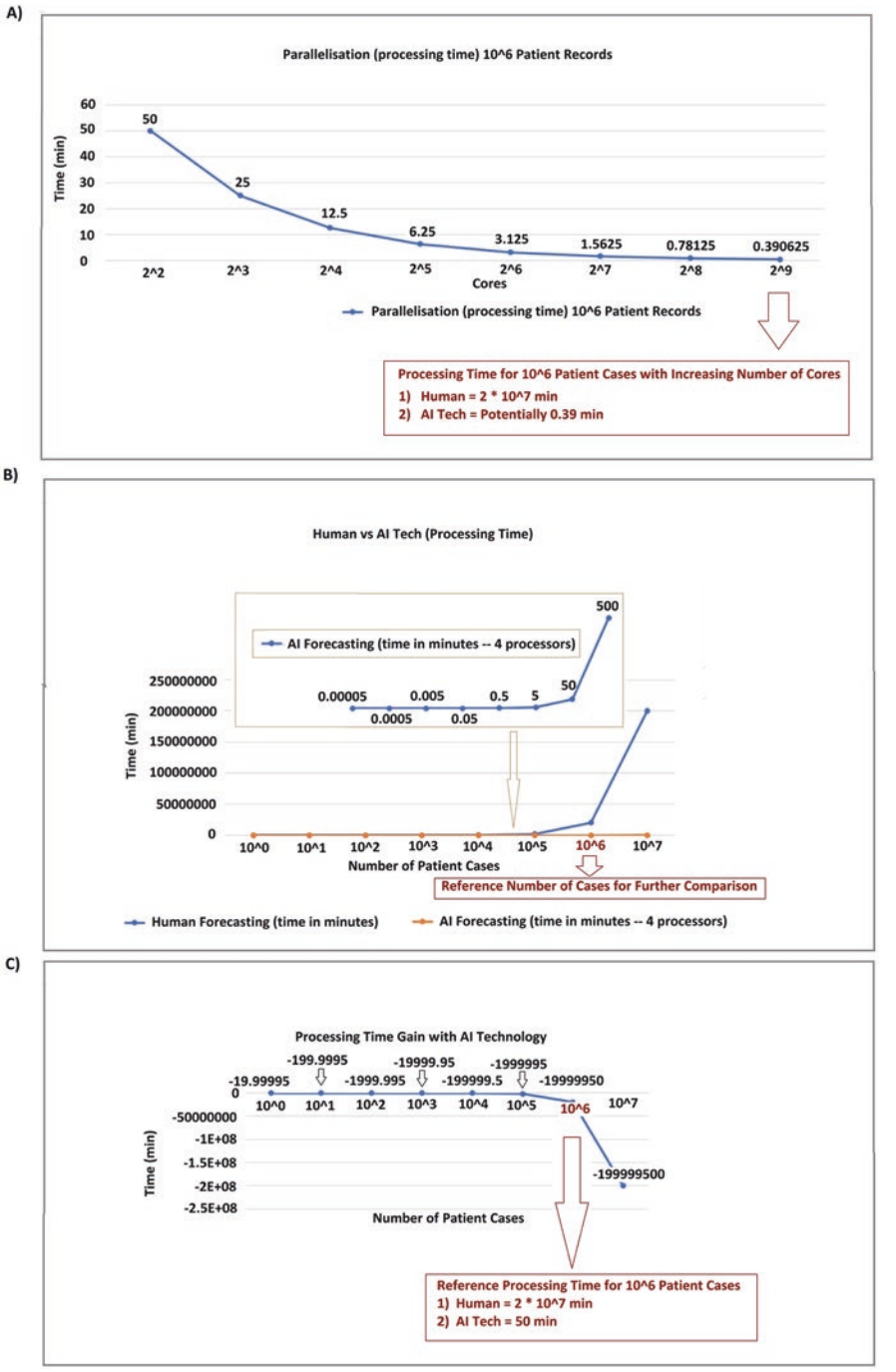

Fig. 4.3 Illustration of AI performance in biomedical forecasting: (**a**) reference time in minutes for AI-based decision-making, with increasing number of cores/processors and increasing number of patient cases, (**b**) camparison between human and AI tech forecasting as a function of

dynamic compound-disease matching (Pereira 2017c; Romano and Tatonetti 2019; Ekins et al. 2019), and optimal dose forecasting in drug delivery, to rapid identification and reconstruction of target signalling pathways in disease progression, along with medical imaging for diagnosis and drug activity follow-up (Pereira 2017c; Shah et al. 2019).

With respect to targeting diseases for drug repurposing, optimised via computational design, neuroinflammation is a multifaceted pathology that can severely compromise the brain, affecting memory and cognitive motor control, causing diseases like brain cancer, Alzheimer's, and dementia. Therefore, neurodegeneration and its inflammatory biomarkers have been the focus of a considerable number of studies (Thackeray et al. 2018; Jayaraj et al. 2019). However, assuming both that immune therapies are being used to knock down resident inflammation in other parts of the human body and that neuroinflammation is commonly related to immune impairment taking place in other organs, the author is of the view that far too little research on drug repurposing for neuro-impairment is found in the current literature. Therefore, in a work published in 2017, the author discussed the correlation between arterial chronic inflammation biomarkers and those used to characterise the progression of glioblastoma multiform (Pereira 2017a), indicating the potential for drug repurposing. The formation of glioblastoma multiform, which according to the World Health Organization (WHO) is a terminal grade 4 type of brain tumour, is significantly influenced by immune responses. In Pereira (2017a), epigenetics is exemplified under the perspective of DNA methylation, which reduces cell differentiation potential. Increasing cell proliferation is debated and associated with overexpression of epidermal growth factor (EGF) receptor/HER1 common variants. Moreover, NFkB family members were there presented as modulators of cytokine transcription, regulating chronic inflammatory signalling pathways, along with cellular differentiation, survival, and proliferation. The last is fundamental in proneural cell differentiation into mesenchymal glioblastoma (Abedalthagafi et al. 2018; Behnan et al. 2019). The author concluded that similarities found are of great interest in computational design and personalised medicine. Hence, exploring information about harmful genotype variants associated with glioblastoma and chronic arterial inflammation is proposed, via computational pattern recognition and forecasting tools, to both target chemical compounds for drug repurposing and predict individual response to experimental therapies. Additionally, the author presented artificial intelligence (AI)-based dynamic design of recombinant DNA, to optimise in silico drug discovery and production of plant secondary metabolites.

Evidence presented by Tusi et al. (Tusi et al. 2010) suggests that 1,2,4-triazine derivatives upregulate hemeoxygenase-1, glutamylcysteine synthetase, glutathione peroxidase, and nuclear factor-erythroid 2 p45-related factor 2 (Nrf2). Additionally,

processing time in minutes, and (**c**) benefits of using AI technology in terms of gain in forecasting time. In diagrams **b** and **c**, the author selected 10^6 patient cases as the reference dataset size, for data analysis. An ensemble regression model was employed and forecasting took 50 min (4 cores), against $2*10^7$ min spent in human forecasting (diagram **c**). Computational forecasting time decreased to 0.39 min (diagram **b**), with increasing number of cores (2^9). The biomedical benchmark problem selected in this study was forecasting patient propensity to develop stomach cancer, following proton pump inhibitor treatment, due to *H. pylori* infection

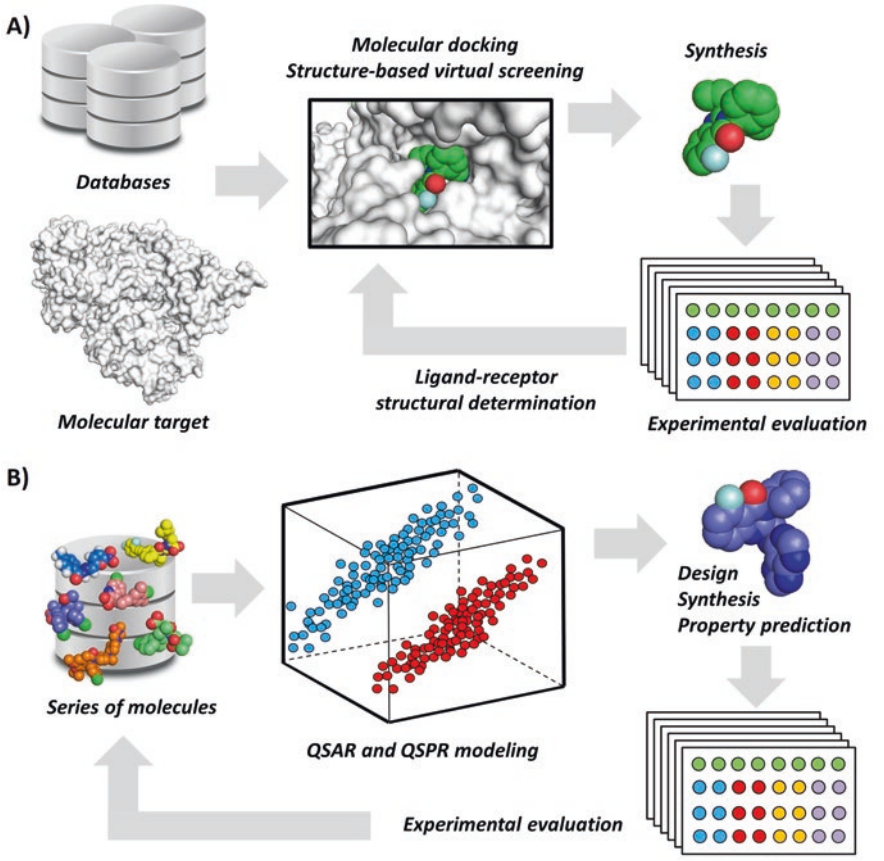

Fig. 4.4 Benefits of chemo-informatics approaches: (**a**) structure-based drug design via virtual screening and molecular docking, which elucidates intermolecular interactions and improves ligand-receptor affinity, among other parameters, and (**b**) illustration of ligand-based drug design, along with both quantitative structure-activity and structure-property relationships, which are used to predict both pharmacodynamics and pharmacokinetic properties [adapted from Ferreira and Andricopulo 2018]

it is indicated that these compounds inhibit NFkB activity and mitigate lipid peroxidation. Altogether, this mitigates apoptosis induced by ROS, in the brain. Ultimately, in that work, the authors show via the application of artificial neural network that 1,2,4-triazine compounds have a great potential to cross the blood-brain barrier, which is mandate for a drug to be effective in treating neurodegeneration. Providing that innovation often involves several disciplines in approaches to topics, it is no surprise that informatics has coupled with chemistry. In chemo-informatics, computing is employed to characterise both molecular constituents and properties of chemical compounds (Fig. 4.4). In the 1990s, the scientific community focused greatly in assays for both generating combinatorial libraries and assessing molecular diversity (Blaney and Martin 1997), matching therapeutic molecular activity and

disease phenotypes. Improving algorithms have motivated further usage of computer science, optimising the structure of compound libraries. Therefore, over the last years, diversity-oriented library design has flourished (Davies and White 2002; Gillet 2002; Lewis 2002; Newton 2002; Roe 2002; Willett 2002). In Kalafatovic et al. (2019), a new approach based on multi-objective genetic algorithms for peptide library design was presented, supported by molecular mass and amino acid diversity. In that work, the authors pursued to optimise tracking amino acid permutations in complex mixtures, using molecular mass to avoid duplicates and sequence overlapping. Their findings indicated that diversity is a fundamental parameter when designing libraries for discovering biologically active peptides, overcoming issues with library size.

Computing-driven data mining, correlation, and database annotation were used in Lacroix et al. (2018), to elucidate polyphenol-protein interactome. As a result, the authors clustered polyphenols and polyphenol-rich food on the basis of their effects in specific metabolic signalling cascades. That work is important to support phenol selection driven by pathophysiology. Indeed, the selective intake of polyphenol-rich nutrients is proven to downregulate both glucaemia and cholesterolaemia (Chew et al. 2019). *Urera aurantiaca* extracts are potent anti-inflammatory and antioxidants (Marrassini et al. 2018). Neuro-impairment driven by oxidative stress and inflammation is apparently attenuated by *Magnolia officinalis* extracts (Chuang et al. 2013), because these compounds modify ionotropic glutamate receptor agonist N-methyl-D-aspartate (NMDA) signalling cascades in microglial cells activated by interferon-γ (IFNγ) and lipopolysaccharide (LPS). Additionally, analysis based on a mouse model of post-traumatic osteoarthritis indicates that green tea phenols are chondroprotective (Leong et al. 2014). At last, in Ngoua-Meye-Misso et al. (2018), *Scyphocephalium ochocoa* was screened for assessing its phenolic content, correlating phenols' classes with reducing angiogenesis, inflammation, and oxidative stress, which are factors found to be influencing chronic inflammatory diseases and cancer. Altogether, the above indicates the synergetic power of employing computational methods in biomedicine.

4.6 Conclusions

Investigations on major biomarkers in the progression of both chronic inflammation and autoimmune diseases have serious implications in elucidating immune impairment and how abnormal signalling cascades may result in both initiation and progression of inflammatory pathologies. Chronic inflammation is in the current literature heavily associated with both NOS and ROS production, resulting in imbalanced redox (Tedgui and Mallat 2006; McInnes and Schett 2007; Di Paolo and Shayakhmetov 2016; Wang et al. 2016, b; Pereira 2017a, b; Liu et al. 2017; Waters et al. 2019; Li et al. 2019; Murphy 2019; Du et al. 2019; Ayala et al. 2019; Sharma et al. 2019; Henrik Heiland et al. 2019; Furiati et al. 2019; Park et al. 2019; Engelbertsen et al. 2019; Insuela et al. 2019; Hwang et al. 2019). Multiple analysis revealed that growth factors, enzymes, lipoproteins, and cytokine families are the main modulators of

inflammatory dysfunction, which commonly leads to a burden of diseases like atherosclerosis and mesenchymal glioblastoma (Vignali et al. 2008; Van Tits et al. 2011; Pereira et al. 2014; Di Paolo and Shayakhmetov 2016; Pereira 2017a, 2017c; Henrik Heiland et al. 2019). Remarkably, finding consensus on the characterisation of certain inflammatory affections within autoimmunity is a challenge, because making predictions about autoimmunity-driven disease initiation may not be straightforward, by lacking sufficient conclusive evidences on causes of illness initiation. Over the last years, the author investigated autoimmunity, in the context of atherosclerosis. Here, fundamental arguments advocating in favour of a positive correlation between cell damage due to redox impairment and autoimmunity driving atherosclerosis initiation are presented, highlighting DNA damage, cell cycle arrest, alterations in apoptosis, and damage to the cell membrane induced by high ROS levels. These findings contribute in several ways to our understanding of atheroma formation and provide the foundations for tracking related autoimmune processes.

In this chapter, the author pursued to illustrate and debate polyphenol-driven modulation of inflammatory signalling pathways in chronic inflammation and autoimmune pathologies. Therefore, polyphenols mitigating harmful levels of oxidative stress were addressed. These insights add to the rapidly expanding fields of drug discovery, repurposing, and herbal therapy development. Phenols' anti-inflammatory and antioxidative properties have been deeply debated, from production of dicarbonyl like the 1,4-benzoquinone (Dangles 2012) to modulation of hydroquinone-based proelectrophilic small molecule-based activation of Nrf2 and HSF-1 pathways (Satoh et al. 2015). Additionally, the author discussed polyphenols as fundamental elements in reducing the concentration of both activated T helper cells (Th1/Th17) and T cell proliferation (Hammer et al. 2017), and modulating chronic inflammatory signalling cascades (Pereira 2017c). Ultimately, the author highlights the analysis of phenols' properties undertaken in the current literature, which has implicitly extended our knowledge of neurodegenerative processes—e.g. examining neuronal dysfunction as the amyloid-β (Aβ) neurotoxicity induced by ROS formation, causing neuronal cells' death due to glutathione deficiency, in Alzheimer's disease (Cao et al. 2018).

The trends here discussed can be used to develop targeted interventions. Therefore, this chapter is concluded with a debate on computational methods leveraging therapeutics, presenting recent findings in computational automation in targeting new metabolites in personalised medicine (Peng et al. 2019; Bezerra-Filho et al. 2019; Wang et al. 2019a), creating screening libraries to optimise genotype-phenotype matching in drug discovery and repurposing (Pereira 2017c; Aulner et al. 2019), and improving drug delivery systems via nanocomposites.

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Plant Alkaloids: Structures and Bioactive Properties

5

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Abstract

Alkaloids are nitrogen-containing natural products found in bacteria, fungi, animals, and plants with complex and diverse structures. The widespread distribution of alkaloids along with their wide array of structures makes their classification often difficult. However, for their study, alkaloids can be classified depending on their chemical structure, biochemical origin, and/or natural origin. Alkaloids can be derived from several biosynthetic pathways, such as the shikimate pathway; the ornithine, lysine, and nicotinic acid pathway; the histidine and purine pathway; and the terpenoid and polyketide pathway. Traditionally, plant alkaloids have played a pivotal role in folk medicines since ancient times as purgatives, antitussives, sedatives, and treatments for a wide variety of ailments. Currently, several alkaloids have served as models for modern drugs, and there are several alkaloids used in pharmacology, such as codeine, brucine, morphine, ephedrine, and quinine. Herein, this work is a comprehensive revision from the Web of Knowledge and Scopus databases on the recent information (2010–2019) regarding plant-derived alkaloids, their structural classification and bioactive properties.

Keywords

Alkaloids · Medicinal plants · Anticancer · Phytochemicals · Natural compounds

5.1 Introduction

Plants are the source of many metabolites with different physiological functions. Among the most studied metabolites are the phytochemicals, which are mainly secondary metabolites produced by plants that vary in structure, amount, location, and activity even in plants from the same cultivar. These metabolites are often classified into three major groups: phenolic compounds, terpenes, and nitrogen- and sulfur-containing compounds (Mazid et al. 2011; Ncube et al. 2015). Alkaloids are a very wide group of natural compounds derived from secondary metabolism, and their main characteristic feature is the presence of a basic atom of nitrogen in any position of the molecule (does not include nitrogen in an amide bond or peptide). They are commonly isolated from plants; however, they have also been found in animals, insects, marine invertebrates, and some microorganisms (Lu et al. 2012; Roberts

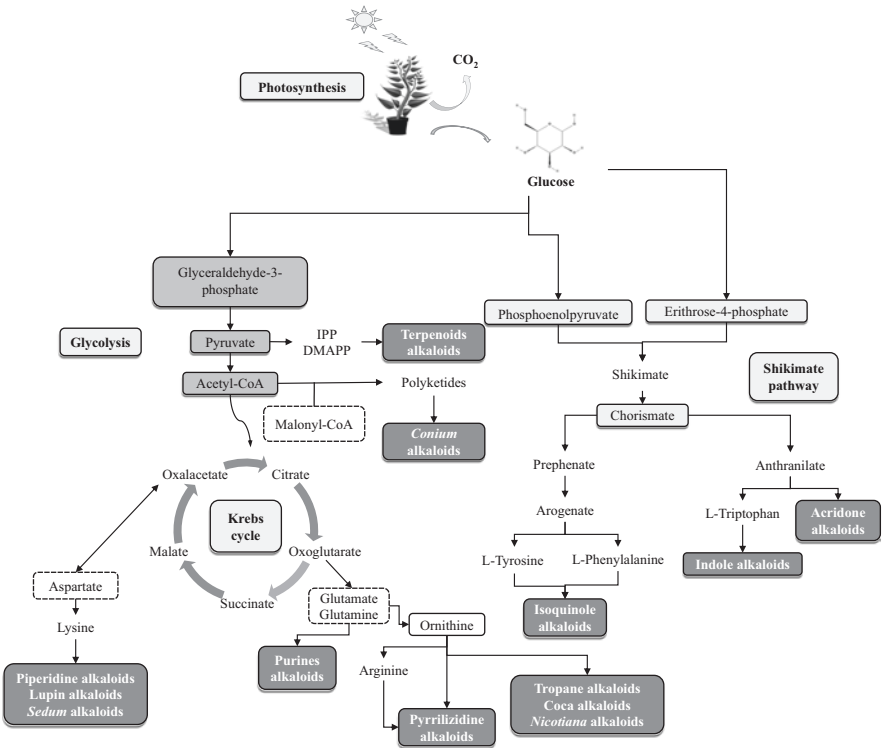
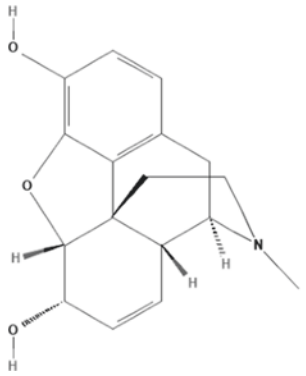


Fig. 5.1 Metabolic pathway of alkaloid biosynthesis. Abbreviations: *IPP* isopentenyl diphosphate, *DMAPP* dimethylallyl diphosphate. Elaborated from data in Wink (2010)

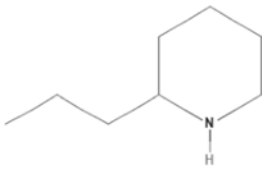
2013; Bribi 2018). They can be present in different organs of the cell like mitochondria, vesicles, chloroplasts, and vacuoles. Its precursors (mainly amino acids) are derivatives of metabolic pathways, such as glycolysis. Figure 5.1 exemplifies how the biosynthetic pathways are as diverse as the group of alkaloids, for example, the aromatic amino acids phenylalanine, tyrosine, and tryptophan precursors of some alkaloids, such as indole and isoquinoline alkaloids, derived from the shikimate pathway (Wink 2010; O'Connor 2010; Aniszewski 2015).

These compounds are abundant in nature, i.e., they are found in at least 25% of plants. They are generally produced to facilitate the survival of plants in the ecosystem, because they are allelopathic compounds, i.e., they have the potential to be a natural herbicide (Jing et al. 2014). Alkaloids are compounds that can be found in plants, fungi, bacteria, and animals. Their function in plants is not entirely understood, but it is highly related to seed formation and protection against predators. Since alkaloid function is not exclusive to the organism producing it, their pharmacological properties have been highly studied (Mazid et al. 2011).

The term alkaloid was originally used to refer to base-type compounds that contained nitrogen and react with acids forming salts. The word is derived from the



Morphine structure. Adapted from PubChem (National Center for Biotechnology Information 2019b)



Coniine structure. Adapted from PubChem (National Center for Biotechnology Information 2019a)

Fig. 5.2 Chemical structure of morphine and coniine (National Center for Biotechnology Information 2019a, b)

word *al-qali*, with an Arabic origin. The study of this group of metabolites began in 1806, when morphine (Fig. 5.2) was isolated from opium by Sertürner. Since then, many plant extracts have been used as poisons and medicine, due to their alkaloid content. Chemically they are defined as crystalline, colorless substances with bitter taste that can form salts when being united to acids; in the plants, they can hide in free state, like salts or like N-oxides (Kutchan 1995; O'Connor 2010; Amirkia and Heinrich 2014; Encyclopædia Britannica 2018; Bribi 2018).

History shows us examples of renowned characters that used alkaloid-based extracts, such as Socrates whose death was caused by *Conium maculatum* extract and Cleopatra, who was known for using *Hyoscyamus muticus* to dilate her pupils to achieve a more attractive appearance. In Medieval Europe, *Atropa belladonna* was frequently used by women aiming for the same results as Cleopatra, being the alkaloid, coniine (Fig. 5.2), the responsible compound in this particular case. Later on, in history, its derivatives began to be used during medical examinations for dilating pupils. Tropicamide is another similar example since it has been used for Alzheimer's disease diagnosis (Ncube et al. 2015). Nowadays, research has led to identifying several types of alkaloids in over 4000 different plants. Some plant families are known for their high content, such as the Papaveraceae, Ranunculaceae, Solanaceae, and Amaryllidaceae families (Encyclopædia Britannica 2018).

Alkaloid classification, which will be further explained in this chapter, depends on many characteristics, mainly chemical properties, taxonomy, and botanical and pharmaceutical functions. The most used classification is given by the position of nitrogen and whether if it's part of the ring or not; in this regard, we can identify heterocyclic alkaloids or typical alkaloids and non-heterocyclic or atypical alkaloids (Evans 2009). Also, depending on their biosynthetic pathway, alkaloids can be classified into terpenoid indole alkaloids, benzylisoquinoline alkaloids, tropane alkaloids, and purine alkaloids (Facchini 2001).

Since ancient times, civilizations have used plants (root, leaf, stem, fruit, and seeds) containing alkaloids as remedies (teas, poultices, potions, etc.) to treat some diseases or as poison (Goyal 2013; Roberts 2013; Jing et al. 2014). The biological significance of alkaloids depends on their significant relation with health benefits. Alkaloids are now medically known anesthetics, stimulants, antibacterials, antimalarials, analgesics, antihypertensive agents, spasmolysis agents, anticancer drugs, antiasthma therapeutics, vasodilators, antiarrhythmic agents, etc. These properties, as well as their toxicity continue to be an important research field (Kuete 2014).

5.2 Classification of Plant Alkaloids

Currently, the *Dictionary of Alkaloids* reports more than 40,000 compounds (Buckingham et al. 2010), and many of them are named according to their origin, that is to say the plant and botanical family from which they were isolated (Table 5.1), as well as can also be classified according to their origin (Talapatra and Talapatra 2015). Moreover, due to the wide diversity of alkaloids distributed in plants and the lack of a taxonomic base to define them consistently, their classification can also be based on their natural or biochemical origin or their chemical structure, the latter

Table 5.1 Alkaloid classification according to their origins and sources

Alkaloids	Plant	Family	References
Berberine	*Berberis asiatica*	Berberidaceae	Mazumder et al. (2011).
Anonaine	*Annona squamosa*	Annonaceae	Porwal and Kumar (2015)
Morphine, papaverine	*Papaver somniferum*	Papaveraceae	Baros et al. (2012)
Salsoline	*Salsola kali*	Amaranthaceae	Boulaaba et al. (2019)
Thalfoliolosumine	*Thalictrum foliolosum*	Ranunculaceae	Li et al. (2016)
Galanthamine	*Galanthus woronowii*	Amaryllidaceae	Bozkurt et al. (2017)
Lycorine	*Amaryllis belladonna*	Amaryllidaceae	Tallini et al. (2017)
Srilankine	*Alseodaphne semecarpifolia*	Lauraceae	Thakur et al. (2012)
Vincristine, vinblastine	*Vinca rosea*	Apocynaceae	Adewusi and Afolayan (2010)
Lupanine	*Lupinus angustifolius*	Fabaceae	Jansen et al. (2012)
Quinine	*Cinchona pubescens*	Rubiaceae	Noriega et al. (2015)
Huperzine A	*Huperzia serrata*	Lycopodiaceae	Ferreira et al. (2016)
Geissospermine	*Geissospermum vellosii*	Apocynaceae	Mbeunkui et al. (2012)
Solanine	*Solanum melongena*	Solanaceae	Friedman (2015)
Piperine	*Piper nigrum*	Piperaceae	Li et al. (2011)
Atropine	*Atropa belladonna*	Solanaceae	Koetz et al. (2017)
Cocaine	*Erythroxylum coca*	Erythroxylaceae	Jirschitzka et al. (2012)
Nicotine	*Nicotiana tabacum*	Solanaceae	Baranska et al. (2013)
Echimidine	*Echium hypertropicum*	Boraginaceae	Carvalho et al. (2013)
Caffeine	*Camellia sinensis*	Theaceae	Wang et al. (2011)

being the most used, and is based on its main structure, i.e., a CN skeleton (Cushnie et al. 2014; Bribi 2018). In this regard, according to their molecular structure and biosynthetic pathway, alkaloids can be divided into three different types: (a) true alkaloids (heterocyclics), (b) protoalkaloids (non-heterocyclics), and (c) pseudoalkaloids (Ranjitha and Sudha 2015).

5.2.1 True Alkaloids (Heterocyclics)

They are chemically complex, physiologically active compounds, and derivatives of cyclic amino acids. They have an intracyclic nitrogen, and can be found in nature forming salts with some organic acids, such as oxalic, lactic, malic, tartaric, acetic, and citric acid (Talapatra, Talapatra 2015; Henning 2013). The alkaloids of this group are derivatives of the amino acids L-ornithine, L-tyrosine, L-phenylalanine, L-lysine, L-histidine, L-tryptophan, L-arginine, and glycine/aspartic acid (Kukula-Koch and Widelski 2017); these amino acids are the basis of a certain group of alkaloids, for instance, tryptophan is the base of the indole, quinoline, and pyrroloindole alkaloids; the amino acid tyrosine is the basis of the isoquinoline alkaloids; the amino acid ornithine for the tropane, pyrrolizidine, and pyrrolidine alkaloids; and lysine for the quinolizidine and piperidine alkaloids; aspartate is the base for the pyridine alkaloids; anthranilic acid is the precursor for quinazoline, quinoline, and acridone alkaloids; and the derivatives of histidine are the imidazole alkaloids (Böttger et al. 2018; Kaur et al. 2019; Aniszewski 2015). The heterocyclic alkaloids are divided into pyrrole, pyrrolidine, pyrrolizidine, pyridine, piperidine, tropane, quinolone, isoquinoline, aporphine, quinolizidine, indole, indolizidine, and imidazole (Fig. 5.3). Within these groups, we can find alkaloids, such as berberine, salsoline, geissospermine, piperine, nicotine, lobeline, nantenine, cocaine, quinine, dopamine, and morphine (Hussain et al. 2018).

5.2.1.1 Pyrrole Alkaloids

This is the most important group of heterocycle alkaloids, since they are present in various natural and unnatural compounds with pharmacological properties. The most commonly known pyrrole alkaloids are the derivatives of heme and chlorophyll. These compounds contain four groups of pyrrole joined by methine bridges (Estévez et al. 2014). The pyrrole ring is the central nucleus of the carbazole alkaloid structure, which can be found mostly in the Rutaceae family, within the genera *Murraya*, *Glycosmis*, and *Micromelum* (Bauer and Knölker 2012).

5.2.1.2 Pyrrolidine Alkaloids

These types of alkaloids are derivatives of the amino acid L-ornithine, and in some cases, they are derived from arginine and lysine with the addition of acetate/malonate units; they contain rings in their structure. Some pyrrolidine alkaloid types are putrescine, hygrine, and cuscohygrine (Kaur and Arora 2015) and can be found in families such as Fabaceae, Erythroxylaceae, and Moraceae (Kukula-Koch and Widelski 2017).

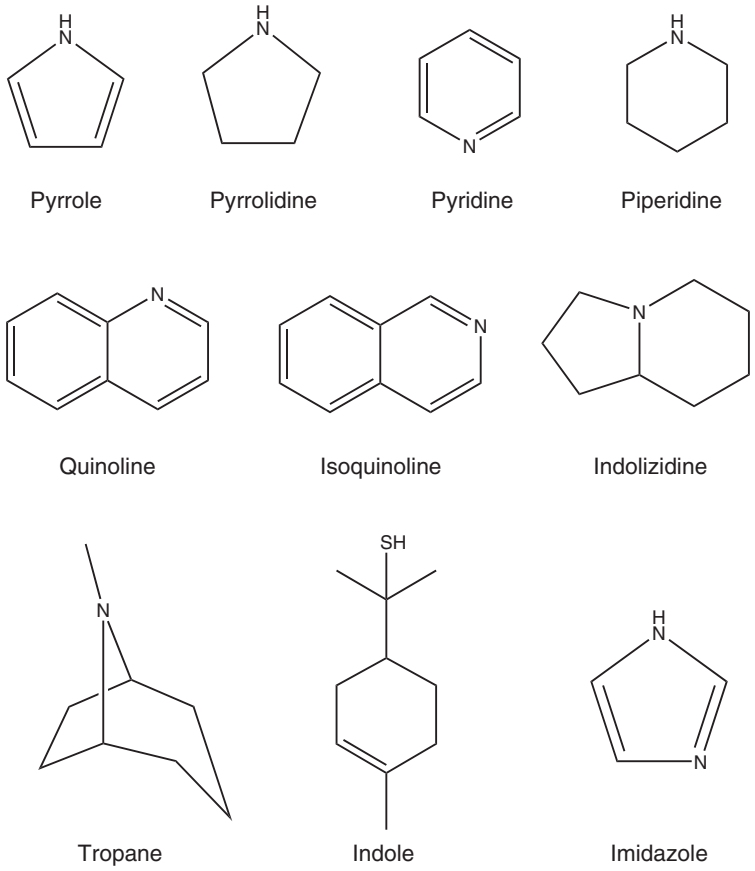


Fig. 5.3 Skeletal structure of true alkaloids (heterocyclics)

5.2.1.3 Pyrrolizidine Alkaloids

More than 500 compounds have been identified as pyrrolizidine alkaloids, which are derivatives of the amino acid ornithine. Pyrrolizidine alkaloids can be found in the form of esters of 1-hydroxymethyl-1,2-dehydropyrrolizidine (necines) and may have a hydroxyl group at position 7; however, the position C-2 and C-6 can be hydroxylated (Koleva et al. 2012; Schramm et al. 2019). They are characterized by having in their structure two rings of five members with an atom of nitrogen between them; a double ligature in C-1 and C-2 determines the toxicity of this alkaloid. According to its base, necines are divided into four groups: retronecine, heliotridine, otonecine, and platynecine (Moreira et al. 2018). Pyrrolizidine alkaloids can be mainly found in the families Asteraceae and Boraginaceae (Koleva et al. 2012).

5.2.1.4 Pyridine Alkaloids

These heterocyclic compounds contain in their nucleus an unsaturated nitrogen radical and are derivatives of the amino acid L-ornithine. Some common pyridine

alkaloids are piperine, coniine, trigonelline, arecoline, arecaidine, guvacine, cytisine, lobeline, nicotine, anabasine, sparteine, and pelletierine (Kaur and Arora 2015). These alkaloids are present in the botanical families Aizoaceae, Annonaceae, Apocynaceae, Araceae, Bignoniaceae, Dipsacaceae, Gramineae, Palmae, and Umbelliferae, among others (Silva Teles et al. 2019).

5.2.1.5 Piperidine Alkaloids

They are widely studied, and about 700 compounds are known as piperidine alkaloids. They are derivatives of L-lysine, and its structure contains a ring of six radicals, five groups of methylene, and one amine. In this group, we can find the alkaloids like solenopsin, cynapine, lobeline, and coniine. Piperidine alkaloids can be found in plants of the family Lobeliaceae, particularly *Lobelia inflata*, which contains the alkaloid lobeline (Liu et al. 2010; Kaur, Arora 2015). We can also find them in black pepper (*Piper nigrum*) as piperine and in *Punica granatum* as pelletierine. Likewise, there are numerous natural products that contain piperidine residues in their skeleton (Goel et al. 2018; Böttger et al. 2018).

5.2.1.6 Quinoline Alkaloids

This group of alkaloids derived from L-tryptophan is heterocyclic aromatic compounds formed by the fusion of a benzene ring with a pyridine ring. Quinine is one of the most important of this group and is found in *Cinchona ledgeriana*, while the quinidine alkaloid is obtained from *C. officinalis*. Some other quinoline alkaloids are pamaquine, chloroquine, tafenoquine, and bulaquine (Marella et al. 2013; Kaur and Arora 2015; Debnath et al. 2018). The plants of the genus *Melodinus* are an important source of these alkaloids, as well as those belonging to the Rutaceae family (Byler et al. 2009; Cai et al. 2011).

5.2.1.7 Tropane Alkaloids

They are derivatives of the amino acid ornithine. Tropane alkaloids have a complex structure and are characterized by their skeleton N-methyl-8-azabicyclo[3,2,1] octane (Mao et al. 2014). More than 200 alkaloids can be classified as tropane alkaloids, and these are mainly distributed in the angiosperms of different families, such as Proteaceae, Solanaceae, Erythroxylaceae, Convolvulaceae, Brassicaceae, and Euphorbiaceae (Jirschitzka et al. 2012). The most studied are found in the Solanaceae family, mostly in the genera *Datura* (hyoscyamine and scopolamine), *Hyoscyamus* (scopolamine), and *Atropa* (atropine alkaloid) (Ajungla et al. 2009; Wink 2010; Guirimand et al. 2010). Another well-known tropane alkaloid is the cocaine, obtained from *Erythroxylum coca* (Erythroxylaceae). Interestingly, 186 alkaloids have been found only in this species (Oliveira et al. 2010; Jirschitzka et al. 2012). Atropine is another common tropane alkaloid and is considered a basic drug list of the World Health Organization (Ranjitha and Sudha 2015).

5.2.1.8 Isoquinoline Alkaloids

They are among the most abundant in the kingdom of plants; we can find them in the families Papaveraceae, Berberidaceae, Fumariaceae, Ranunculaceae, Rutaceae,

Amarryllidaceae, and *Annonaceae* (Kukula-Koch and Widelski 2017). Isoquinoline alkaloids are heterocyclic aromatic compounds derived from the amino acids tyrosine and phenylalanine, formed from 3,4-dihydroxytyramine (dopamine). According to their structure, isoquinoline alkaloids can be divided into simple isoquinolines, which have a benzene ring attached to a pyridine ring, and benzylisoquinolines, which contain a second aromatic ring (Khan and Kumar 2015). Likewise because they are a structurally non-homogeneous group and depend on the degree of oxygenation, intramolecular rearrangement, distribution, and the presence of additional rings that are connected to the main system, these can be divided into subgroups benzylquinoline, aporphine, protoberberine, benzo[c]phenanthridine, protopine, phthalide isoquinoline, morphine, and emetine alkaloids (Bhadra and Kumar 2011; Koleva et al. 2012; Kukula-Koch and Widelski 2017; Hussain et al. 2018). One of the most known alkaloid is morphine, which is an isoquinoline alkaloid, and is obtained from opium (*Papaver somniferum*). In this species, we also find the alkaloid codeine, a benzylisoquinoline alkaloid; berberine in *Berberis aristata*, *B. lyceum*, and *B. tinctoria*; and colchicine in *Colchicum autumnale* (Baros et al. 2012; Diamond and Desgagné-Penix 2016; Debnath et al. 2018).

5.2.1.9 Aporphine Alkaloids

Aporphine alkaloids are heterocyclic alkaloids derived from isoquinoline alkaloids. These compounds have been isolated from approximately 100 genera and from diverse families (approximately 20), such as Annonaceae, Menispermaceae, Papaveraceae, Ranunculaceae, Lauraceae, Monimiaceae, Magnoliaceae, and Berberidaceae, among others. Some common aporphine alkaloids are caaverine, lirinidine, asimilobine, N-methyl-asimilobine, nornuciferine, nuciferine, anonaine, magnoflorine, dicentrine, boldine, galucine, and neolitsine. All of the aforementioned differ in the substituents in the position of the nitrogen atom; these can be -H, -CH3, -COOH3, etc. (Chen et al. 2013; Muthna et al. 2013).

5.2.1.10 Quinolizidine Alkaloids

These compounds are derivatives of the amino acid L-lysine and are formed by the fusion of two rings of six members that share a nitrogen atom, and its structural variation goes from simple to complex. We can find them mainly in the Leguminosae family, especially in the genus *Lupinus*; however, they are also present in the genera *Baptisia*, *Thermopsis*, *Genista*, *Cystus*, and *Sophora.* The most commonly known quinolizidine alkaloids are lupanine (*Lupinus luteus*), cytisine (*Laburnum* species), and sparteine alkaloids (*Sarothamnus scoparius*) (Bunsupa et al. 2012; Szőke et al. 2013; Kaur and Arora 2015).

5.2.1.11 Indole Alkaloids

They have a bicyclic structure, consisting of six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring, the latter providing the property of basicity, and its precursor is the amino acid tryptophan (Hamid et al. 2017). There are approximately 2000 identified compounds known as indole alkaloids distributed in different families such as Apocynaceae, Loganiaceae, Rubiaceae,

and Nyssaceae, as well as plant species such as *Catharanthus roseus* (vinblastine and vincristine alkaloids), *Rauvolfia serpentina* (ajmalicine), *Camptotheca acuminata* (camptothecin), *Passiflora incarnata* (harman, harmol, harmine, harmaline), *Mitragyna speciosa* (mitragynine), *Rauwolfia serpentina* (reserpine, serpentinine, ajmaline, corynanthine, and yohimbine (Guirimand et al. 2010; Sagi et al. 2016; Hamid et al. 2017).

5.2.1.12 Indolizidine Alkaloids

The precursor of indolizidine alkaloids is the amino acid L-lysine. Indolizidine alkaloids are heterocyclic alkaloids, characterized by the fusion of six- and five-membered rings, with a nitrogen atom between ring fusions. They have been found in different plants of the genus *Ipomoea*. Specifically, the alkaloids like ipalbine, ipalbidine, and isoipomine occur in *I. alba* (seeds). Among others, *I. carnea* possesses the alkaloid swainsonine, which is found in most of the plants of this genus; however, it was initially isolated from plants of the genus *Swainsona* (Meira et al. 2012; Diaz 2015). The main indolizidine alkaloids are swainsonine, castanospermine, and lentiginosine (Michael 2008).

5.2.1.13 Imidazole Alkaloids

These alkaloids are derived from L-histidine, and this group is comprised of the alkaloids histamine, histidine, pilocarpine, and pilosine, obtained mainly from two families Cactaceae and Rutaceae (Aniszewski 2015). Pilocarpine is the main imidazole alkaloid and was isolated from leaves of *Pilocarpus microphyllus* of the Rutaceae family; some other alkaloids of this group have been isolated from this plant, for example, isopilosine, epiisopilosine, and epiisopiloturine (Silva et al. 2013; Debnath et al. 2018).

5.2.2 Protoalkaloids (Non-heterocyclics)

Chemically, protoalkaloids contain the nitrogen atom outside the ring, which remains as part of a side chain, not as part of the heterocyclic system; it can be derived from amino acids or from biogenic amines. Some protoalkaloids are mescaline, ephedrine, colchicine, cathinone, etc.; however, they are not so common in nature (Wansi et al. 2013; Talapatra and Talapatra 2015; Jayakumar and Murugan 2016; Kukula-Koch and Widelski 2017). Protoalkaloids can be derived from L-tyrosine and L-tryptophan, which derive into phenylethylamine and terpenoid indole, respectively (Alves de Almeida et al. 2017). Mescaline is one of the most common phenylethylamine alkaloids and can be obtained from *Lophophora williamsii*, commonly known known as peyote (Beyer et al. 2009). On the other hand, monoterpenoid indole alkaloids are a fairly large group in which around 3000 have been identified in families as Apocynaceae, Loganiaceae, and Rubiaceae (Pan et al. 2016).

5.2.3 Pseudoalkaloids

Pseudoalkaloids are heterocyclic containing nitrogen compounds but are not derived from amino acids; they are generally derivatives of acetate, pyruvic acid, adenine/guanine, or geraniol (Aniszewski 2015). As an example of this group, we can mention the diterpenoid alkaloids (of 18, 19, and 20 carbons) obtained from a variety of sources such as the genus *Aconitum*, *Consolida*, and *Delphinium* (Wang et al. 2010; Gao et al. 2012).

5.3 Plant Sources of Alkaloids

A plant is considered as a source of alkaloids when the species contain more than 0.001% of alkaloids (Wang and Liang 2009). In the present section of this chapter, we present a summarized compilation of botanical families of plants which are sources of alkaloids of pharmacological importance.

5.3.1 The Amaryllidaceae Family

Amaryllidaceae is a family of monocotyledonous plants with significant economic importance for its horticultural and ornamental appeal as well as its medicinal value. The family Amaryllidaceae is composed of about 1100 species in 75 genera that are distributed in warm tropical and subtropical zones around the world, including South America, the Mediterranean, and Southern Africa (Nair et al. 2013). The leaves are fleshy and two-ranked with parallel veins with linear, strap-like, oblong, elliptic, lanceolate, or filiform shape. The flowers are bisexuals and typically arranged in umbels, and the fruit is dry and capsule shaped or fleshy and berry-like. More than 100 alkaloids have been isolated from Amaryllidaceae plants, and some of them are shown in Table 5.2, which exert a wide range of interesting physiological effects.

Table 5.2 Plant alkaloids from the Amaryllidaceae family

Plant	Common name	Alkaloids	References
Crinum powelli	Cape lily	Lycorine, 1-O-acetyl lycorine Ismine	Nino et al. (2007)
Hippeastrum puniceum	Easter lily	Didehydroanhydrolycorine, lycorine, narciclasine, pancratistain	Cortes et al. (2015) Santana et al. (2008)
Lycoris radiata	Hurricane lily	Dehydrodihydrolycorine, 6β-acetoxycrinamine, O-acetylhomolycorine, N-oxide, caranine, ungerine, homolycorine	Feng et al. (2011) Huang et al. (2013)
Hippeastrum vittatum	Bulb mavem	Montanine, lycorine, vittatine, vittacarboline, ismine, O-methylamine, pancracine, hippadine	Silva et al. (2008) Youssef (2001)

5.3.2 The Apocynaceae Family

Plants of the Apocynaceae family, commonly known as oleander or dogbane family, are most commonly found in tropical and subtropical regions and have ornamental value. These plants are well known for their alkaloid content. The Apocynaceae family is composed of around 200 species and about 2000 genera of trees, shrubs, herbs, and lianas or vines sometimes succulents or cactus-like. The herbs, shrubs, and trees have opposite leaves and a milky, latex sap (Joselin et al. 2012). The flowers are bisexual and regular, with five united sepals, five united petals, and five stamens; stamens attach at the base of the petals, alternate with the lobes. Some typical genera of the Apocynaceae family includes *Angadenia*, *Apocynum*, *Asclepias*, *Catharanthus*, *Ceropegia*, *Cynanchum*, *Gonolobus*, *Hoya*, *Mandevilla*, *Morrenia*, *Secamone*, and *Vallesia*, many of which are poisonous; however, in correct dosage, they are useful in current medicine (Table 5.3).

Table 5.3 Plant alkaloids from the Apocynaceae family

Plant	Common name	Alkaloids	References
Alstonia angustiloba	Red-leafed pulai	Yohimbine, cathafoline, cabucraline, vincamajine, normacusine B, lochnerine, alstophylline, macralstonine, villalstonine, alstilobanine	Ghedira et al. (1998) Ku et al. (2011)
Alstonia scholaris	White cheese wood	Echitamine, tubotaiwine, akuammicine, echitamidine, alstonamine, rhazmanine, strictamine, manilamine, angustilobine, vallesamine, tubotaiwine	Roy (2015) Macabeo et al. (2005)
Catharanthus roseus	Madagascar periwinkle	Catharanthine, vindoline, vincristine, serpentine, vinblastine, ajmalicine	Hisiger and Jolicoeur (2007)
Cynanchum paniculatum	Swallow-wort root	Antofine	Lee et al. (2003)
Holarrhena floribunda	Kurchi bark	Holarrhesine; holadiene; conessine	Hoyer et al. (1978)
Ochrosia elliptica	Elliptic yellow wood	Ellipticine; methoxy ellipticine; elliptinine; isoreserpiline; cathenamime; pleiocarpamine; apparicine; epchrosine; tetrahydroalstonine	Chen et al. (2017)
Plumeria alba	Caterpillar tree Pagoda tree Pigeonwood	Curine; evonine; voacamine; tubocurarine chloride; syrosingopine	Sibi et al. (2014)
Rauwolfia vomitoria	Poison devil's pepper	Sarpagan; picrinine; akuammiline; heteroyohimbine; yohimbine; aricine; isoreserpiline; rauvoxine; rauvoxinine	Patel et al. (1964)
Rauvolfia caffra	Quinine tree	Strictamine; sarpagan; akuammicine; indolenine; corynane; peraksine; yohimbine; suaveoline; heteroyohimbine; oxindole	Milugo et al. (2013)
Tabernaemontana divaricata	Pinwheel flower	Cononitarine B; conophylline	Zalaludin (2015)

5.3.3 The Papaveraceae Family

Plants from the Papaveraceae family are dicotyledonous flowering plants that possess leaves lobed or dissected, with bisexual flowers in color red, white, violet, or yellow and fruits in the form of capsules with dark seeds. Papaveraceae plants contain about 42 genera which includes *Canbya*, *Argemone*, *Corydalis*, *Romneya*, *Arctomecon*, *Stylomecon*, *Mechonopsis*, *Hunnemannia*, *Dendromecon*, *Eschscholzia*, *Meconella*, *Fumaria*, *Glaucium*, *Macleaya*, *Sanguinaria*, and *Papaver*, among others, with 775 species, distributed mainly in the subtropical and temperate regions of northern hemisphere (Xu and Deng 2017). Papaveraceae plants are strong narcotics, and also have biological and medical importance. Papaveraceae plants contain L-tyrosine-derived alkaloids such as morphine, codeine, and noscapine from *Papaver somniferum* and protopine, isocorydine, stylophye, rhoeadine, coptisine, and tetrahydropalmatine from *Papaver rhoeas*. These have irreplaceable therapeutic value in the treatment of many diseases. In addition, different alkaloids belonging to this family have been reported (Table 5.4).

5.3.4 The Asteraceae Family

The Asteraceae family is one of the largest families of flowering plants, consisting of approximately 1600 genera and over 23,000 species with a variety of morphological traits from trees of up to 30 m tall and small herbs of approximately 1 cm height; flowering heads occur in an amazing range of colors, sizes, and shapes (Bohm and Stuessy 2001), unisexual or bisexuals, sometimes sterile calyx reduced, corolla flattened, or tubular, and leaves alternate, opposite, or whorled exstipulate.

Table 5.4 Important alkaloids from plants of Papaveraceae family

Plant	Common name	Alkaloids	References
Tabernaemontana divaricata	Pinwheel flower	Voaphylline	Zalaludin (2015)
Glaucium flavum	Yellow horned poppy	Glaucine, talikmidine, isocorydine, norisocorydine	Petitto et al. (2010)
Glaucium grandiflorum	Red horned poppy	Corydine, isocorydine, oxoglaucine, pontevedrine	Kintsurashvili and Vachnadze (2000)
Chelidonium majus L.		Chelidonine, chelerythrine, sanguinarine, isochelidonine	Ciric et al. (2008)
Argemone mexicana	Flowering thistle	13-Oxoprotopine, protomexicine, dehydrocorydalmine, jatrorrhizine	Singh et al. (2016)
Chelidonium majus	Swallowwort	Chelidonine, berberine, coptisine, sanguinarine, chelerythrine	Gañán et al. (2016)
Sanguinaria canadensis	Bloodroot	Sanguinarine, chelerythrine, chelilutine, sanguirubine, chelirubine, allocryptopine	Croaker et al. (2016)

Table 5.5 Alkaloids of Asteraceae family

Plant	Common name	Alkaloids	References
Ageratum conyzoides	Billy goat weed Chickweed	Lycopsamine, echinatine	Wiedenfeld and Roder (1991)
Centaurea montana	Mountain bluet	Montanoside	Shoeb et al. (2006)
Centaurea schischkinii		Schischkiniin, montamine	Shoeb et al. (2005)
Senecio cineraria	Silver dust	Senecionine, seneciphylline, integerrimine, jacobine, jacozine, jacoline, jaconine, otosenine, florosenine, floridanine, doronine	Tundis et al. (2007)

The most common genera are *Ambrosia*, *Artemisia*, *Aster*, *Bidens*, *Centaurea*, *Cirsium*, *Elephantopus*, *Gaillardia*, *Helianthus*, *Jurinea*, *Liatris*, *Rudbeckia*, *Senecio*, and *Vermonia* (Table 5.5).

5.3.5 The Solanaceae Family

The Solanaceae family contains 90 genera and more than 2000 species distributed in all continents and are abundant in alkaloids. Some typical genera are *Brugmansia*, *Atropa*, *Datura, Physalis*, *Mandragora*, *Solanum*, *Petunia*, *Nicotiana tabacum*, *Physalis*, *and Lycium.* Plants from the Solanaceae family can take the form of herbs, shrubs, trees, and vines, and sometimes epiphytes, and can be annuals, biennials, or perennials, and some have tubers. They do not produce latex or colorless saps. The leaves are generally alternate or alternate at the base of the plant and opposed toward the inflorescence, and the leaves can be herbaceous, leathery, or transformed into spines. The plant species belonging to this family grow especially in the tropic and subtropics. The majority of the species occur in Central and South America. Hyoscyamine, hyoscine, and cuscohygrine are tropan alkaloids from *Atropa belladonna* L. (Pérez-Amador et al. 2007). Some species with important alkaloid content are listed in Table 5.6.

5.3.6 The Rutaceae Family

The citrus botanical family (*Rutaceae*) is distributed across tropical and subtropical areas. Some species such as *Dictamnus albus*, *Skimmia japonica*, and *Acronychia baueri* contain the anthranilic acid-derived alkaloids such as dictamnine, skimmianine, and acronycine, respectively. Alkaloids derived from L-histidine such as pilocarpine and pilosine are present in the species *Pilocarpus microphyllus* and *P. jaborandi* (Santos, Moreno 2004). Non-citrus fruits with alkaloids include white sapote, orangeberry, clymenia, and limeberry (Table 5.7).

Table 5.6 Alkaloids from plants of the Solanaceae family

Plant	Common name	Alkaloids	References
Datura innoxia	Downy thorn apple	Acetylcopine, atropine, scopolamine, hysoscyamine	El Bazaoui et al. (2012)
Duboisia myoporoides	Corkwood	Scopolamine, hysoscyamine, butropine, apoatropine	Palazón et al. (2003)
Nicotiana glauca	Tree tobacco	Anabasine, nornicotine	Mizrachi et al. (2000)
Physalis minima	Ground cherry	Phygrine, withaminimim	Basey et al. (1992)
Solanum dulcamara	Bittersweet	Solanine, solasodine, solamarine	Kumar et al. (2009)
Solanum nigrum	Black nightshade	Solasodine	Jiang et al. (2006)
Solanum torvum	Turkeyberry	Solasodine, solasonine, solamargine	Pérez-Amador et al. (2007)

5.3.7 The Fabaceae Family

This plant family is the third largest botanical family and is rich with alkaloids derived from L-ornithine and L-tryptophan such as eserine, eseramine, physovenine, and geneserine which can be found in *Physostigma venenosum*. Also alkaloids from Fabaceae family are derived from L-tyrosine like jasmonoyl-S-dopa and N-jasmonoyl isolated from *Vicia faba*; and alkaloids are derived from L-lysine like lupinine, sparteine, lupanine, angustifoline, epilupinine, and anagyrine, among others (Table 5.8). Plants of the Fabaceae family grow in humid tropics, subtropics, and temperate and subarctic regions around the globe, with 18,000 species and 650 genera, among them *Acacia*, *Albizia*, *Baptisia*, *Cassia*, *Cercis*, *Dalea*, *Delonix*, *Erythrina*, *Glycine*, *Mimosa*, *Parkia*, *Phaseolus*, etc. (Kramell et al. 2005).

5.3.8 The Rubiaceae Family

The Rubiaceae family is characterized with flowering plants known as bedstraw family. They are terrestrial trees, shrubs, lianas, or herbs. It is the fourth largest angiosperm family that contains about 13,500 species in 611 genera, such as *Acranthera*, *Aidia*, *Aidiopsis*, *Airosperma*, *Alberta*, *Anthorrhiza*, *Appunia*, *Badusa*, *Benkara*, *Bobea*, *Borojoa*, *Bouvardia*, *Breonadia*, *Capirona*, *Calycosia*, *Canthium*, *Ceriscoides*, *Chassalia*, *Chione*, *Cigarilla*, *Coffea*, *Coptosapelta*, *Cowiea*, *Cuviera*, *Cubanola*, *Danais*, *Dichilanthe*, *Deppea*, *Geophila*, *Gouldia*, *Greenea*, *Haldina*, *Hamelia*, *Phitopis*, *Pinckneya*, *Pimentelia*, *Pomax*, *Praravina*, *Pseudopyxis*, *Ramosmania*, *Rennellia*, *Rubia*, *Rustia*, *Sacosperma*, *Schachtia*, *Saldinia*, *Serissa*, *Simira*, *Sinoadina*, *Sommera*, *Stevensia*, *Stipularia*, and *Suberanthus*, among others. Most of its species that are present in the subtropical regions belong to *Cinchona* (source of quinine) and have high commercial values (Table 5.9) (Nadkarni et al. 1995). Due to

Table 5.7 Alkaloids from Rutaceae family

Plant	Common name	Alkaloids	Reference	Alkaloids	References
Aegle marmelos	Bael tree	Aegeline, marmeline, shahidine, skimmianine, ethyl cinnamamide	Sugeng et al. (2001) Yadav and Chanotia (2009)	Aegeline, marmeline, shahidine, skimmianine, ethyl cinnamamide	Sugeng Riyanto et al. (2001); Yadav and Chanotia (2009)
Casimiroa edulis	White sapote	Edulein, scopoletin, zapoterin, casimiroedine	Awaad et al. (2012)	Edulein, scopoletin, zapoterin, casimiroedine	Awaad et al. (2012)
Evodia rutaecarpa	Wu Zhu Yu or Evodia fruit	Evodiamine, rutaecarpine, evocarpine, 1-methy-2-[(6Z,9Z)]-6,9-pentadecadienyl-4-(1H)-quinolone (IV), 1-methyl-2-dodecyl-4-(1H)-quinolone (V)	Jiang and Hu (2009)	Evodiamine, rutaecarpine, evocarpine, 1-methy-2-[(6Z,9Z)]-6,9-pentadecadienyl-4-(1H)-quinolone (IV), 1-methyl-2-dodecyl-4-(1H)-quinolone (V)	Jiang and Hu (2009)
Skimmia japonica	Japanese *skimmia*	Skimmianine	Sackett et al. (2007)	Skimmianine	Sackett, Towers, and Isman (2007)
Toddalia asiatica	Orange tree	8-Methoxynorchelerythrine, 11-demethylrhoifoline B, 8-methoxynitidine, 8-acetylnorchelerythrine, 8,9,10,12-tetramethoxynorchelerythrine, isointegriamide, 1-demethyl dicentrinone, 11-hydroxy-10-methoxy-(2,3),methylenedioxytetrahydroprotoberberine, nitidine, magnoflorine, 8-methoxynitidine	Hu et al. (2014)	8-Methoxynorchelerythrine, 11-demethylrhoifoline B, 8-methoxynitidine, 8-acetylnorchelerythrine, 8,9,10,12-tetramethoxynorchelerythrine, isointegriamide, 1-demethyl dicentrinone, 11-hydroxy-10-methoxy-(2,3),methylenedioxytetrahydroprotoberberine, nitidine, magnoflorine, 8-methoxynitidine	Hu et al. (2014)

Table 5.8 Alkaloids from Fabaceae family

Plant	Common name	Alkaloids	References
Albizia gummifera	Peacock flower	Budmunchiamine K Budmunchiamine G Normethylbudmunchiamine K	Rukunga and Waterman (1996) Mahlangu et al. (2017)
Erythrina variegata	Tiger claw	Spirocyclic (6/5/6/6) erythrivarine A (1), spiro-fused (6/5/7/6) rings erythrivarine B	Suryawanshi and Patel (2011) Zhang et al. (2014)
Sophora flavescens	Shrubby sophora	12α-Hydroxysophocarpine, oxymatrine, matrine, 9α-hydroxymatrine, allomatrine, oxysophocarpine, sophocarpine, anagyrine, 9α-hydroxysophocarpine, lehmannine, 13,14-dehydrosophoridine	Ding et al. (2006)

Table 5.9 Important alkaloids from Rubiaceae family

Plant	Common name	Alkaloids	References
Cinchona officinalis	Cinchona bark	Quinine, quinidine, cinchonine, cinchonidine	Song (2009)
Mitragyna speciosa	Tang	Corynoxine, mitragynine, speciogynine, paynantheine, corynoxine B	Poklis and Peace (2017)
Nauclea orientalis	Bur tree	Naucleficine; naucleactonine; naucleaorals	Sichaem et al. (2012) Zhang et al. (2001)
Psychotria colorata		Calycanthine, isocalycanthine, chimonanthine, hodgkinsine, quadrigemine C	Verotta et al. (1998)
Uncaria tomentosa	Cat's claw	Pteridine, speciophylline, isomitraphylline, uncarine F, mitraphylline, isopteropodine	Sandoval et al. (2002)

the increasing studies claiming potential human health promotion effects, alkaloids have been gaining popularity around the world and are currently being used as major therapeutic agents (Table 5.10).

5.4 Bioactive Properties of Plant Alkaloids

Plant alkaloids have been used as medicines, since ancient times, and their use is widespread around the world. The ethnobotanical use of alkaloid-rich plants led the way to elucidate, isolate, and evaluate the pharmacological properties of these compounds that have ended in the production of several drugs, which are being used at

Table 5.10 Anticancer properties of plant alkaloids

Alkaloid type	Alkaloids	Anticancer effect	References
Vinca alkaloids	Vinblastine, vincristine, vindesine, and vinorelbine	Antitumor activity against MCF-7, MDA-MB-231, HepG2, HepG2/ADM, and K562 cells	Zheng et al. (2013)
Mitragyna speciosa	Tang	Corynoxine, mitragynine, speciogynine, paynantheine, corynoxine B	Poklis and Peace (2017)
Nauclea orientalis	Bur tree	Naucleficine; naucleactonine; naucleaorals	Sichaem et al. (2012) Zhang et al. (2001)
Psychotria colorata		Calycanthine, isocalycanthine, chimonanthine, hodgkinsine, quadrigemine C	Verotta et al. (1998)
Uncaria tomentosa	Cat's claw	Pteridine, speciophylline, isomitraphylline, uncarine F, mitraphylline, isopteropodine	Sandoval et al. (2002)

present. Alkaloids have been described with many uses; nowadays they are used as chemotherapy agents, and also are being studied for their antidiabetic and neuroprotective capacity.

5.4.1 Anticancer Properties of Plant Alkaloids

One of the main challenges in cancer treatment is the development of multidrug resistance to chemotherapy agents, which is caused mainly by the efflux of the drugs by the p-glycoprotein (Joshi et al. 2017). Plant alkaloids are of interest in plant medicinal chemistry and medicine due to their ability to act as anticancer agents in some drug resistant-cancer types. Alkaloids also have been suggested for the prevention and/or management of oxidative stress and inflammation, both related to cancer (Alasvand et al. 2019). Vinca alkaloids, such as vinblastine, vincristine, vindesine, and vinorelbine, have shown antitumor activity against breast cancer cells like MCF-7 and MDA-MB-231, hepatic cancer cells (HepG2, HepG2/ADM), and leukemia cell line (K562). Their antitumor effect is hypothesized through the electron-withdraw substituents on the ring, which may be associated with their potential tubulin-binding activity (Zheng et al. 2013). Drug resistance is a common problem during chemotherapy treatment, and some cell lines resistant to some chemotherapeutic agents have shown sensibility to treatment with alkaloids such MCF-7_{TXT}, a docetaxel-resistant cell line, with the alkaloid colchicine in a study by Wang et al. (2017). The authors also reported that the cell line MCF-7_{TXT} showed higher cytotoxicity against the alkaloids vinorelbine and vinblastine than the non-resistant MCF-7 cc cell line. Moreover, the authors showed that the docetaxel-resistant MCF-7 cells were cross-resistant to vinca alkaloids, but sensitive to colchicine, 2MeOE2, ABT-751, and CA-4P, which are microtubule-targeting

agents, that are considered as one of the most reliable classes of anti-neoplastic drugs in the treatment of breast cancer. As aforementioned, drug resistance is a persistent problem during chemotherapy treatment in cancer patients. For example, during pemetrexed therapy, the enzyme thymidylate synthase levels are enhanced in cancer tissue of nonsquamous non-small cell lung cancer. Chiu et al. (2017) reported that vinca alkaloids in in vivo studies with vinblastine and in vitro studies with vincristine can successfully inhibit the growth of pemetrexed-resistant tumors. Vinca alkaloids regulate the ERK-mediated pathway, which is a regulator of apoptosis induced by pemetrexed. Moreover, one of the most common and important classes of anticancer agents are tubulin-binding compounds, which interfere with microtubule assembly leading to mitotic arrest. However, most of these compounds are cytotoxic; thus new compounds from natural origin are being studied. For instance, vinblastine, vincristine, vindesine, and vinorelbine are known antimitotic drugs, and Zheng et al. (2013) showed that the structural differences among these compounds affect their antitumor properties and their toxicity. The authors reported that these alkaloids showed moderate antitumor activity in MCF-7, MDA-MB-231, HepG2, HepG2/ADM, and K562 cell lines; and this effect is suggested to be mediated by electron-withdraw substituents from the ring structure. Another molecular mechanism related to tumor cell growth is the interaction between high-mobility group box 1 protein and receptor for advanced glycation end products. The high-mobility group box 1 protein is a nonhistone DNA-binding protein involved in inflammation, cell migration, cell death, and tumor metastasis with high affinity to receptors for advanced glycation end products, which are related to inflammation, tumor cell growth, migration, and invasion (Sims et al. 2010). In this sense, Inada et al. (2019) evaluated the potential of the alkaloid papaverine as anticancer agent in human glioblastoma (GGB) temozolomide-sensitive U87MG and TMZ-resistant T98G cells and reported that, in fact, papaverine can prevent tumor growth promotion by inhibition of the high-mobility group box 1 protein in human glioblastoma temozolomide-sensitive U87MG and TMZ-resistant T98G cells. Additionally, a study by Xie et al. (2019) with amide alkaloids from *Piper nigrum* showed that dimeric alkaloids such as pipernigramide A; pipernigramide B, an unknown new identified natural compound; chabamide; chabamide I; nigramide B; and piperine enhanced the sensibilization of paclitaxel-resistant cervical cancer cells HeLa/PTX. These alkaloids significantly enhanced the anticancer apoptotic effect of paclitaxel. Furthermore, the combination treatment of *P. nigrum* alkaloids at 50 μM and paclitaxel enhanced the apoptotic effect through increased cleavage of PARP and caspase-3. The alkaloid piperine enhanced the response of paclitaxel-resistant cervical cancer cells; this effect was mediated by a change in the expression of molecules related with the apoptotic pathway. Furthermore, combination treatment decreased the protein expression of phosphor-Akt and Mcl-1, which are involved in the paclitaxel resistance process in cancer cells. It has been reported that the antioxidative transcription nuclear factor Nrf2 increases during tumor malignancy in cancer cells such as colonic, thyroid, endometrial, lung, breast, and pancreatic cancer cells. This may cause alterations that may affect the genetic material, such as loss-of-function mutations and promotion of hypermethylation, and also chemoresistance. Since some

alkaloids have shown potential to be Nrf2 inhibitors, Arlt et al. (2013) evaluated the suppressive effect of trigonelline on Nrf2 activity in pancreatic cancer cells. The authors showed that trigonelline exerted high inhibitory effect on Nrf2 activity at doses from 0.1 to 1 μM; interestingly higher doses were less efficient/ineffective. The alkaloid trigonelline decreased the nuclear level of Nrf2 protein but not its overall expression (Arlt et al. 2013). Amusingly, no adverse effects have been reported for trigonelline in human studies.

Alkaloids are currently promising compounds to be used in cancer treatment, alone or in combination with other anticancer therapies. However, further studies are still necessary to fully understand their molecular and cellular mechanism of action as well as their toxicity and pharmacological properties.

5.4.2 Antidiabetic Properties of Plant Alkaloids

Several reports have shown promising studies on the antidiabetic properties of alkaloids from different plants, such as *Rhizoma coptidis*, *Trigonella foenum-graecum*, *Berberis vulgaris*, and *Ervatamia microphylla*, which have been reported to exert their antidiabetic potential through several mechanisms such as diminishing insulin resistance, promoting insulin secretion, and ameliorating gut microbiota structures, among others (Zhou et al. 2012; Pirillo and Catapano 2015; Mirhadi et al. 2018; Umezawa et al. 2018; Ma et al. 2019).

For instance, *Coptis chinensis* alkaloids like berberine, epiberberine, coptisine, palmitine, and magnoflorine have been related with anti-obesity effects. Choi et al. (2014) showed that *C. chinensis* alkaloids inhibit adipogenesis in 3 T3-L1 cells; alkaloid action was dose dependent without any apparent cytotoxic effect. The authors suggest that the potential obesity-ameliorating effect of *Coptis* alkaloids is through the downregulation of major adipogenic transcription activators such as PPAR-γ and C/EBP-α proteins. Further, in vivo research and clinical trials are needed to clarify the efficacy, safety, and precise molecular mechanisms of the anti-obesity effects of these alkaloids.

Plant alkaloids can also be potential antidiabetic agents by their potent α-glucosidase inhibitory activity. Choudhary et al. (2011) showed that nummularine-R, nummularin-C, and hemsine-A cyclopeptide alkaloids isolated from *Ziziphus oxyphylla* Edgw are potent α-glucosidase inhibitor with IC_{50} values of 212.1, 215.1, and 394.0 μM, respectively and that the alkaloids nummularine-R and hemsine-A are also anti-glycation agents. Moreover, Choi et al. (2015) reported that alkaloids from rhizome of *Coptis chinensis*, identified as berberine, epiberberine, magnoflorine, and coptisine, possess antidiabetic effect mediated by their inhibitory potential against protein tyrosine phosphatase 1B, a non-transmembrane protein tyrosine phosphatase, an enzyme in which its overproduction is involved in the onset of non-insulin-dependent diabetes mellitus. The authors reported that the evaluated alkaloids had inhibitory activities against PTP1B with IC_{50} vales of 16.3, 24.19, 28.14, and 51.04 μM, respectively, and that this inhibition was mixed-type for berberine and epiberberine and noncompetitive for magnoflorine and coptisine. Furthermore, a docking simulation analysis showed that the

evaluated alkaloids have high proximity to PTP1B residues, including Phe182 and Asp181 in the WPD loop, Cys215 in the active sites, and Tyr46, Arg47, Asp48, Val49, Ser216, Ala217, Gly218, Ile219, Gly220, Arg221, and Gln262 in the pocket site, which indicates a higher affinity and tighter binding capacity of these alkaloids for the active site of the enzyme. Another study by Hulcova et al. (2018) showed that Amaryllidaceae alkaloids are potential glycogen synthase kinase 3β inhibitors. Twenty-eight alkaloids of seven structural types (1) belladine, (2–6) haemanthamine, (7–10) crinine, (11–13) galanthamine, (14–19) lycorine, (20) tazettine, and (21–28) homoycorine were reported by the authors. Only caranine, 9-O-demethylhomolycorine, and masonine showed glycogen synthase kinase 3β inhibitory activity above 50%. Interestingly, the two homolycorine-type Amaryllidaceae alkaloids, masonine and 9-O-demethylhomolycorine, and one lycorine-type alkaloid caranine showed the highest IC_{50} values with 27.81, 30, and 30.75 μM, respectively. Interestingly, the authors described a detailed structure-activity relationship where the presence of hydroxyl substitution at position 2, as in hippeastrine, is connected with a distinct reduction of GSK-3β inhibitory activity compared with masonine, 9-O-demethylhomolycorine, oduline, and O-ethyllycorenine, where no substituent in position C-2 is present. The opening of the tetrahydropyrane ring in tetrahydromasonine also reduces the GSK-3β inhibitory potency of homolycorine-type alkaloids. However, further structure-activity relationship studies are needed. Moreover, Ullah et al. (2018) reported that streptozotocin-induced diabetic rats treated with steroidal alkaloids from *Sarcococca saligna* at a subcutaneous dose of 5 mg/kg reduced the glucose level in blood. This effect was attributed to the alkaloids sarcovagine-D and holaphylline, and also these alkaloids were related with the good improvement in blood lipids. It is important to mention that abnormal lipid levels in diabetic patients may produce hypertriglyceridemia and high cholesterol in blood. A 4-week study by Zhang et al. (2018) showed that alkaloids from *Litsea glutinosa* barks in ob/ob mice at doses of 50, 100, and 200 mg/kg decreased body and fat weights without reducing average food intake in treated mice; the efficiency of the treatment was similar to that of metformin. The identified alkaloids in the extracts used in the treatment were laurelliptine, 6-isoquinolinol, laurolitsine, isoboldine, N-methyl laurolitsine, laurolitsine, boldine, and litseglutine. Furthermore, the *L. glutinosa* alkaloid extracts at concentrations ranging from 100 to 200 mg/kg significantly reduced the serum levels of fasting glucose, glycosylated hemoglobin, and glycosylated serum protein. The authors also showed that the alkaloid extract from *L. glutinosa* significantly enhanced the activity of liver glucokinase, a key enzyme in glycogen synthesis, and increased the content of hepatic glycogen. Moreover, a chronic inflammation is a common characteristic of diabetes, which may lead to insulin resistance; in this regard, the alkaloid treatment significantly decreased the inflammation markers such as MCP-1, TNF-α, and IL-6. The vindoline, vindolidine, vindolicine, and vindolinine, alkaloids from the dichloromethane extract of *C. roseus*, induced high glucose uptake in pancreatic β-T6 cells at a concentration of 25 μg/ml. Furthermore, the alkaloids vindolidine, vindolicine, and vindolinine demonstrated inhibitory activity against tyrosine phosphatase 1B. Also, *C. roseus* alkaloids showed a higher antioxidant activity than quercetin, which may be involved in controlling oxidative stress damage caused by ROS production, and is related with the onset of diabetes comorbidities such as cardiovascular

problems like atherosclerosis (Tiong et al. 2013; Halliwell and Gutteridge 2015). Alkaloids also have the potential to be antidiabetic agents due to their modulation of blood glucose and lipid content. For example, *Capparis decidua* alkaloids attenuated the activity of glucose 6-phosphatase by 44% in streptozotocin-induced diabetic mice. Moreover, liver and muscle glycogen content also improved by 33 and 28%, respectively, with alkaloid treatment. In this regard, the lipid profile of alkaloid-treated mice as well as their level of total cholesterol, low-density lipoprotein, and triglyceride decreased around 25, 32, and 27%, respectively. On the other hand, the level of high-density lipoprotein improved by 28% with alkaloid treatment (Sharma et al. 2010). The authors also evaluated the mechanism of action by evaluating the expression profiles of genes involved in glucose homeostasis from alkaloid-treated mice. Expression of glucose regulatory genes, G6Pase and PEPCK, reduced clearly in the treated group. On the other hand, hepatic GK and Glut-4 expression improved significantly in comparison to the diabetic untreated group. Also, streptozotocin-induced upregulation of TNF-α in adipose tissue was significantly downregulated by the alkaloid treatment. Moreover, transcription of PPAR-α gene also increased in the adipocytes of the treated diabetic mice; there was almost no change in the level of PPAR-α. Interestingly, alkaloids managed to reduce renal aldose reductase expression in treated animals as compared to untreated mice.

5.4.3 Plant Alkaloids and Alzheimer's Disease

Several plant alkaloids are of interest due to their potential to be used as drugs to treat neurodegenerative disorders such as Huntington disease, Parkinson's disease, epilepsy, schizophrenia, and Alzheimer's disease (Hussain et al. 2018). Alzheimer's disease is one of the major neurodegenerative diseases and is characterized by progressive deterioration of memory, learning, and other cognitive functions. The main hallmarks of Alzheimer's disease are the accumulation of amyloid plaques containing extracellular deposits of β-amyloid peptide and intraneuronal neurofibrillary tangles, which lead to neuronal cell loss in the nucleus basalis of Meynert and in the hippocampus (Konrath et al. 2013; Hussain et al. 2018). Furthermore, normal cells have a neuronal microtubule-associated protein called tau protein to stabilize the axonal microtubules; however, in Alzheimer's disease, this protein becomes hyperphosphorylated by kinases disassociating it, thus destabilizing the microtubule network, cytoskeletal collapse, loss of viability and neuronal cell death. Interestingly, the β-amyloid peptide accelerates tau protein aggregation, and reduction of the β-amyloid peptide expression may block the amyloid-induced neuronal dysfunction (Ng et al. 2015). In this regard, alkaloids may have a role in Alzheimer's disease, since they have the potential to be good inhibitors of acetylcholinesterase, a key enzyme in the breakdown of acetylcholine, involved in Alzheimer's disease (Konrath et al. 2013).

As it has been established, the chemical structure of compounds with bioactive properties on health promotion influences heavily on their activity. In this regard, a study by McNulty et al. (2010) showed that the inhibition of alkaloid-1-acetyllycorine,

a potent Amaryllidaceae alkaloid with acetylcholinesterase inhibition capacity, functions through their ability to act as hydrogen bond acceptor, analogous to the –OH group of galanthamine, and also the introduction of lipophilic substituents at C-1 and C-2 plays a pivotal role on their acetylcholinesterase inhibitory potential. Also, the distribution of alkaloids in each plant source heavily influences their potential bioactive effect, which may be the result of a synergistic effect or by a single compound. On this subject, Cardoso-Lopes et al. (2010) reported that the solvent of choice to extract alkaloids from plants has a significant effect on their inhibitory rate on the acetylcholinesterase enzyme, which, as aforementioned, may be related to the alkaloid composition in each solvent fraction. The authors used ethanol, hexane, and alkaloid fractions of *Esenbeckia leiocarpa*, which showed acetylcholinesterase inhibitory rates with IC_{50} values of 50.7, 6.0, and 1.6 μg/mL, respectively. This inhibitory activity was related with the presence of alkaloids such as leiokinine A, leptomerine, kokusaginine, skimmianine, masculine, and flindersiamine. Furthermore, Zhan et al. (2010) isolated indole alkaloids from *Ervatamia hainanensis* to identify the compound responsible for the acetylcholinesterase inhibitory activity. They reported that the alkaloids coronaridine and voacangine have the same level of acetylcholinesterase inhibitory potency as galantamine, a known inhibitor, with IC_{50} values of 8.6 and 4.4 μM for coronaridine and voacangine, respectively. Interestingly, voacangine is an analog of coronaridine, with a methoxyl at phenyl group displayed nearly twofold improvement in acetylcholinesterase potency compared to coronaridine. Neuroinflammation is a pathological hallmark of Alzheimer's disease. In this sense, microglial cells (specialized macrophages found in the nervous central system) are activated by amyloid β peptide to produce increased amounts of proinflammatory molecules such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, nitric oxide (NO), and reactive oxygen species. Furthermore, *Ligusticum chuanxiong* is ethnobotanically used in oriental medicine to treat cardiovascular and cerebrovascular diseases, and some studies have shown antioxidant and anti-inflammatory effects, and these properties have been linked to the alkaloid tetramethylpyrazine. On this subject, the alkaloids tetramethylpyrazine have been able to suppress the activity of $A\beta_{1–42}$-induced proinflammatory mediators and neurotoxicity; thus tetramethylpyrazine is a potential alkaloid that can be used as treatment for neurodegenerative diseases like Alzheimer's disease (Kim et al. 2014). An in vivo study by Chonpathompikunlert et al. (2010) reported that the oral administration of piperine at doses from 5 to 20 mg/kg BW during 2 weeks to Wistar rats, showed that piperine, at all doses, had a neuroprotective effect by measuring mice neuron density in regions of the hippocampus, which resulted in improved memory impairment, decreased escape latency, and increased retention time. However, due to the potential cytotoxicity of piperine, further pre-clinical studies are needed before piperine is used in humans.

Conversely, the use of alkaloids must be cautionary, since they may show toxicity, and toxicity and pre-clinical studies are needed before they are used in humans. For instance, it has been reported that *Areca* nut has been associated with oral and pharyngeal cancers, which is associated with its arecoline content, an alkaloid that has shown to be genotoxic and cytotoxic (Shih et al. 2010). Thus, Shih et al. (2010)

evaluated the mechanism of action of the cytotoxic effect of arecoline in rat primary cortical neurons. They showed that arecoline at concentrations ranging from 50 to 200 μM induced neuronal cell death and increased the production of reactive oxygen species and mRNA levels of NADPH oxidase 2, and also arecoline enhanced the expression of proapoptotic proteins, such as cytochrome C, Bax, caspase-9, and caspase-3. Moreover, the authors also reported that antioxidant enzymes can attenuate the redox disruption caused by arecoline.

5.5 Conclusion and Future Perspectives

Alkaloids are a widespread group of compounds with extended use as medicinal agents throughout the world, since ancient times. The chemical structural diversity, distribution, and functional properties of alkaloids are very complex, as well as their study. However, the importance of alkaloids as potential biopharmaceuticals relies on the fact that nowadays, they are used as chemotherapeutic agents to treat diseases, including cancer, diabetes, and neurological disorders. The search for new alkaloids to treat chemotherapy-resistant cancers still continues. However, due to the wide chemo-diversity of alkaloids, the work to describe their structures and pharmacological properties are still needed alongside pre-clinical and clinical studies to test their safe use in humans.

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Pelargonidin, a Dietary Anthocyanidin in the Prevention of Colorectal Cancer and Its Chemoprotective Mechanisms

6

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Abstract

Diseases of bowl wall mucosa stemming from sudden mutation lead to the development of colorectal cancer (CRC) cells by the transformation of normal epithelial cells into neoplastic lesions. CRC is considered to be a global burden; hence, its incidence rate is expeditiously increased up to ten-fold higher, worldwide. The epidemiological report pinpointed CRC as the utmost third common malignancy in men and second in women. Because of greater efficacy, the synthetic drugs are unsatisfactory due to higher toxic effects to the normal cells, and a chance of developing multidrug resistance by tumor cells. Therefore, dietary flavonoids with potent anticarcinogenic effects have been focused on recent investigations. Pelargonidin (PD), a bioactive molecule classified under anthocyanidin is present in red and pink pigmented berries. PD efficiently modulates intercellular antioxidant status, thereby reducing oxidative DNA damage, cellular proliferation, differentiation, apoptosis, angiogenesis, and reverse drug resistance of metastatic cells, and potentially induces cell cycle arrest, thereby interfering in colorectal carcinogenesis. PD scavenges and normalizes the intracellular reactive oxygen species (ROS), which results in gene mutation and induction of colon carcinogenesis. Therefore, the proliferation of tumor cells would be affected or blocked potentially due to disturbance in cell cycle protein by these ROS. Considering the wide pharmacological benefits of PD, this chapter deliberately reviews the cumulative research data from in vitro human colon cancer cell line studies on chemoprotective property of PD against CRC, and also summarizes the underlying mechanism in experimental models.

Keywords

Colorectal cancer (CRC) · Pelargonidin · Antioxidant · Apoptosis · Chemoprevention

6.1 Introduction

Flavonoids are low-molecular-weight large group bioactive polyphenols with a basic benzo-γ-pyrone as a backbone structure, and are widespread in a variety of plants. These bioactive compounds potentially induce apoptosis and modulate tumor cell proliferation, angiogenesis, and differentiation, thereby directly interfering at each step of carcinogenesis (Ramos 2007). A number of research studies evidenced that

secondary metabolites include flavonoids which are phenolic in nature and possess several pharmacological activities based on its unique chemical structure (Mahomoodally et al. 2005). Basically, the degree of chemical reactions such as hydroxylation, substitutions, polymerization, and conjugations determines its specific chemical properties. More interestingly, the antioxidant, metal chelating, and free radical scavenging efficiency of flavonoids were enumerated by the number of hydroxyl groups present in the flavonoids. Chelating property of flavonoids primarily prevents damage of biomolecule by stress-induced intracellular free radicals. Previous studies had evidence that flavonoids possess a chemoprotective effect against various degenerative and infectious diseases like cancer and cardiovascular diseases. Pelargonidin (PD), a bioactive molecule classified under anthocyanidin, is present in red and pink pigmented berries. This phytochemical efficiently modulates intercellular antioxidant status, thereby reducing oxidative DNA damage, cellular proliferation, differentiation, apoptosis, angiogenesis, and reverse drug resistance of metastatic cells, and potentially induces cell cycle arrest, thereby interfering in colorectal carcinogenesis. PD scavenges and normalizes the intracellular reactive oxygen species (ROS), which results in gene mutation and induction of colon carcinogenesis. Therefore, this chapter aims to provide a cumulative research data from in vitro human colon cancer cell line studies on chemoprotective property of PD against CRC, and also focuses to summarize the underlying mechanism in experimental models.

6.2 Colorectal Cancer (CRC): A General Overview

Rapid growth and progressive spreading through adjoining tissues lead to metastasis, and the formation of secondary tumors at different sites is the basic nature of the tumor cells (Tanaka 2009). Successive angiogenesis and uncontrolled proliferation of stem cells are the two important hallmarks of carcinogenesis (Neergheen et al. 2010). Colorectal cancer can be mentioned as a mucosal disease. Bowel wall mucosal lining is found to be the basal origin of colon cancer. This bowel wall anatomically consists of different layers, such as mucosa, submucosa, the serosa, and the muscularis propria. In particular, single-layered columnar epithelial goblet cells of bowel wall were termed as mucosa, which produces mucus, and identified as the primary site of colon cancer induction due to genetic mutations. The mutated cells in this region usually proliferate very rapidly and spread into the lumen. Further progression takes place at microvilli, where the crypts of Lieberkuhn were reprocessed. The strongest layer stand under mucosa is submucosa, and it is considered as an important region for carcinogenesis, and hence this layer includes vascular blood vessels and terminal and lymphatics nerve fibers by the way the tumor cells invade the circulatory system and spread throughout the body.

6.2.1 Colorectal Cancer Epidemiology

Worldwide, third most frequently recorded cancer in men and the second common cancer in women was statically reported as CRC (Ferlay et al. 2015). The incidence

rate increased up to ten-fold higher worldwide with a steep increase in notable countries, such as Australia, New Zealand, the Czech Republic, Slovakia, Kuwait, Israel, and low middle-income countries. Increased risk factors including an unbalanced diet, increased body weight, alcohol, and habitual tobacco consumption are the major reasons behind the sudden increase in the prevalence of CRC in Asian and Eastern European countries (Torre et al. 2015). Moreover, poor diagnosis at initial stage of CRC would lead to metastasis, and cordial epidemiological report had stated that it would be the universal burden which would record more new colon cancer cases approximately 2.2 million peoples and mortality may reach up to 1.1 million by the year of 2030 (Arnold et al. 2017; Ouakrim et al. 2015). Even though the mortality rates throughout the world have been declining in a huge amount in a number of countries, mortality still occurs in few countries like Romania, South America, Russia, and Brazil (Torre et al. 2015).

6.2.2 Etiology of Colorectal Cancer

The major lifestyle-related risk factors include lack of physical activity, over bodyweight, red meat and canned food intake in large amounts, habitual smoking, consumption of alcohol, and a diet lacking in vegetables and fruits. Previous family or individual history of colon polyps, and individual history of type 2 diabetes, bowel disease due to chronic inflammation, or genetic diseases such as Lynch syndrome or familial adenomatous polyposis has been proved as an important risk factor for CRC. The precise causes of colorectal cancer seem to be unknown. However, few studies state the etiology of developing colorectal cancer. The prevalence of CRC has been gradually increasing in urbanized countries, and also the rate of incidence in economically transitioning countries has been raised due to Western lifestyle culture. High fat, high calories diet, processed meats, and low fiber intake are strongly associated with the development of colorectal cancer. It has been proved that the chemical carcinogen produced while the meat was overcooked at a very high temperature, which includes frying, boiling, and grilling, is prone to cause colon cancer (Gingras and Béliveau 2011). Sedentary lifestyle and obesity play a major part in the development and progression of the number of cancer including CRC were evident by epidemiological reports. Lack of physical activity and increased body weight are the two important modifiable and inter-relatable risk factors that account third of CRC. The physical activity, which stimulates the movement of colon and the passage of waste through the colon activity, is associated with a decreased risk of CRC (Lee et al. 2007). Epidemiological evidence indicates that increased consumption of red meat such as beef, pork, and lamb are prone to cause colorectal cancer in both men and women. Stastical data had shown substantial proportion, ranging from 30% to 70%, of all colorectal cancer cases are attributable to diet with red and processed meat intakes, which is implicated as important dietary risk factors. Red meat, processed meat, and meat derived heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), nitrites, and nitrates are the known animal mutagenic compounds formed when muscle meat is cooked using high temperature. Nitrites are

harmful and are more susceptible to develop cancer at proximal colon, whereas HCAs and PHAs cause cancer in the region of the distal colon and rectum. Greater red meat and processed meat intakes were more consistently associated with colorectal carcinoma (Miller et al. 2013).

6.2.3 Pathogenesis of Colorectal Cancer

In accordance to etiology of CRC, chronic inflammation and sudden mutation resulted in the formation of polyps on distinct sites of colon and rectum, and epithelial lining is the primary characteristic of colorectal cancer development, and these polyps can be nonneoplastic or neoplastic. The microenvironment of cancer cells and epigenetic changes along with genetic mutations play a key role in multistep carcinogenesis. These epigenetic mechanisms mostly programmed in most of the cancer cells in the process of DNA methylation along with alterations in histone proteins (Bardhan and Liu 2013). The CRC development in most of the cases would be a subsequential modulation in signaling pathways as a result of respective oncogenes activation and also inactivation of tumor suppressor genes. In addition, chromosomal gains or losses are termed as chromosomal instability (CIN), and sudden insertion/deletion in the specific DNA sequence would be the reason for microsatellite instability (MSI) (Jass 2007). Most often, etiology of distal colon cancer development and progression was mainly due to CIN, which accounts for about 60–70% of total CRC cases. However, most common proximal colon cancer was observed in MSI cases. Mutations in adenomatous polyposis coli (APC) were induced by dysplastic aberrant crypt foci, where it pawed away in the transformation of polyps to cancer by activation Wnt pathway. Such mutations in APC or genes involved in Wnt signaling pathways such as KRAS or TP53 would help in the progression of dACF to tumor cells (Nosho et al. 2008). Also, epigenetic alterations in the promoter region of the respective gene including DNA methylation and histone modifications is the key factor in cancer cells epigenetic inheritance DNA methylation involves the enzymatic addition of a methyl group to the 5′-position of cytosine by DNA methyltransferases (DNMTs) to form 5-methylcytosine. The DNMTs work on specific CG dinucleotide sequences, known as CpGs. More than 70% of the cytosine (C) bases in the context of CpG dinucleotides are supposed to undergo methylation. The biallelic promoter CpG island methylation of the mismatch repair gene MLH1 is characterized to be the primary epigenetic mechanism leading to the development of sporadic colorectal cancer associated with MSI-H (Bardhan and Liu 2013). Histone deacetyl transferases (HDACs) are the most widely characterized proteins among the histone modification enzymes that play predominant roles in the development of CRC. The high HDAC expression has been shown to be associated with reduced survival in CRC patients (Ashktorab et al. 2009).

Intracellular oxidative stress is mainly because of the imbalanced state of unstable highly reactive free radicals and the intermediate metabolites. These free radicals were commonly called reactive oxygen species (ROS), and also the inbuilt cellular mechanism to neutralize and protect cells from such ROS is termed as

antioxidants. ROS rapidly attacks the bricks of the cellular components such as all the important biomolecules that cause serious damage and defect to the whole organism (Ďuračková 2010). Intensive studies on oxidative stress mechanism during the last 20 years by a number of researchers have unmasked its key role in chronic inflammation; thereby, it has a significant role in the progression of several inflammation-related disease conditions, including cancer, cardiovascular, diabetes, pulmonary, and neurological diseases. When cells encounter a sudden attack by oxidative stress, it triggers several subsequent inflammation-related molecular pathways, such as NF-κB, HIF-1α, AP-1, PPAR-γ, p53, β-catenin/Wnt, and Nrf2 (Reuter et al. 2010). Widely occuring procarcinogens, the toxin from environmental pollution, and intracellular phagocytosis by chemo drugs generate an enormous amount of ROS as the metabolic intermediates (Klaunig et al. 2011). Oxidative stress has been found to take part in the aging process, and also it has a major impact on the prevalence of cancer in old age. More interestingly, any serious damage to the genomic DNA or mitochondrial DNA due to ROS will imitate carcinogenesis in most cases. Further, such a process may vary based on the type of tissue or triggered/suppressed transcription of certain gene or modulation in the signaling pathway or some mutational errors in replication, which would significantly result in genetic instability. It has also been studied that a notable amount of intracellular ROS was generated in tumor cells which leads to a specific microenvironment that maintains constant oxidative stress in tumor cells which favors cancer progression. Human cancer is a major activator of antioxidant defense mechanisms. With respect to its apoptosis-inducing capacity, molecules that promote ROS production may be novel therapeutics, proposed to selectively target cancer cells by elevating ROS levels beyond a tolerable threshold to induce mitochondrial outer membrane permeabilization (MOMP) and cell death (Graham et al. 2010).

Most of the common physical work and intermediates of cellular metabolism results in the inevitable production of oxidants and free radicals. At the same time, an inbuilt protective mechanism for the human body is antioxidants and that will be different for each cell type and tissues, and its mode of action might vary as a synergistic effect or even antagonistic effect based on the site of action. The cellular antioxidant defense system includes natural enzymatic antioxidant, namely catalase (CAT) which hydrolyzes H_2O_2, superoxide neutralizing enzyme superoxide dismutase (SOD), glutathione-dependent catalytic enzymes glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), and nonenzymatic antioxidants such as retinol (Vit A), ascorbic acid (Vit C), and tocopherol (Vit E) which includes vitamin A, vitamin C, vitamin E, polyphenols, pigmented carotenoids, and other natural by-products as antioxidants, paying attention great interest in recent decades. Antioxidants at very low concentrations have a higher efficacy to prevent any oxidative damage to cells when compared to antioxidants that donate an electron to stabilize free radicals and thereby inhibit its detrimental effects. SOD is an endogenous antioxidant that involves catalysis dismutation reaction, thereby producing hydrogen peroxide (H_2O_2) from most of the superoxides (O_2^-). Further, CAT catalyzes H_2O_2, the end product of SOD, and splits them into oxygen and water. On the other hand, glutathione-dependent enzyme glutathione peroxidase

(GPx) significantly neutralizes the transition metals, and with the help of GSH as cofactor, it also catalyzes H_2O_2 in the aqueous phase of the cell membrane (Valadez-Vega et al. 2013). GR catalyzes the reduction of oxidized glutathione and thereby maintains the cellular GSH level, thus altering the GSH/GSSH ratio. Further, GSH and intracellular electrophile conjugation were facilitated by glutathione-S-transferase (GST), also called Phase II detoxifying enzymes. The conjugation of GSH to electrophilic compounds is catalyzed by Phase II detoxifying enzymes called glutathione-S-transferase (GST). GSH has been considered as an important natural intracellular antioxidant and has a major role in neutralization and detoxification of ROS such as peroxides, which is produced as a result of increased LPO. It also takes a role in conjugation and excretion of toxic molecules, and thereby it maintains the normal cell structure and function (Dickinson and Forman 2002).

Human beings are constantly bombarded by ROS by means of exogenous factors, such as tobacco, smoking, chemicals, ultraviolet (UV) rays, and many other agents. It also includes drugs or medicines which are used for medical practice. The aerobic cellular metabolism generates highly reactive free radicals in terms of oxidants, which cause serious damage to different types of cells and tissues due to normal physiological conditions. The protection mechanism against infection involves NADPH enzyme activity found in neutrophils and macrophages, and also the electron transfer system of mitochondria or microsomes and peroxisomes takes a vital role in the production of ROS endogenously (Noda and Wakasugi 2001). ROS are not only prone to cause cancer but also many other human diseases (Hippeli et al. 1999). The necrotic lesion was formed by the peroxidation of the biological membrane by highly reactive oxygen with lipids (Halliwell 1994). These ROS in higher amounts alter the biomolecules, such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA). The active oxygen species also induce gene mutation, which results in the development of cancer; on the other hand, it also modulates signal transduction and either triggers/suppresses transcription factors which are also a mechanism of induction of carcinogenesis (Floyd et al. 1986). Tumor suppressor genes p53 and gene involved in cell cycle regulation are highly susceptible to mutations as a result of DNA damage in the presence of ROS. Also the product of LOP, malondialdehyde, potentially induces mutation. Many researchers have pointed out the necessity of enzymatic and nonenzymatic antioxidants to maintain the natural oxidative homeostasis of the organism especially in tumor therapy. Endogenous enzymatic antioxidants, such as SOD, CAT, GPx, GR, and GSt, play a vital role in chelating superoxides and peroxides. Therefore, tumor therapy is basically based on oxidative stress and antioxidant homeostasis (Čipak gašparović et al. 2010).

Plant products are widely used in the present world as medicines, due to the fact that they are very effective therapeutically. Moreover, they are relatively more safe when compared to any synthetic drugs (Singh and Tripathi 2018; Datir 2018; Akhtar and Swamy 2018). Natural products, mainly plant metabolites, exert superior antitumor properties by reducing the action of reactive oxygen species and protecting the critical constituents of cells, that is, macromolecules like nucleic acids and proteins from oxidative damages (Ravichandra et al. 2018). As a result, plant-derived metabolites have attracted researchers in the field of natural medicines to design effective chemodrugs for

treating cancers. Thus, plant-derived compounds are well recognized as anticancer agents, and some of them are being used in the present market for treating various cancer types (Akhtar and Swamy 2018). Drug molecules like camptothecin, vincristine, and vinblastine are some of the examples of chemodrugs derived from plant sources. However, these drugs pose a challenge of toxicity and cause side effects. Hence, a continuous search is in progress to innovate novel drug molecules against cancers. In this regard, different plant species are being explored to isolate new phytochemicals and to evaluate their bioactivities. Likewise, plant molecules are also evaluated for their antiproliferative potentials. Pelargonidin, an anthocyanidin, is among them, and the details of it are given in the following section. Also, the role of a phytochemical against different CRC cells is discussed, emphasizing on its mechanisms of actions.

6.3 Pelargonidin: An Anthocyanidin

The chemical structure of anthocyanins consists of flavylium cation, which is linked to hydroxyl (–OH) and/or methoxyl ($–OCH_3$) groups along with one or more sugar molecules. Anthocyanins without sugars are known as anthocyanidins. Six major anthocyanidins which are abundant in fruits and vegetables are pelargonidin, malvidin, delphinidin, cyanidin, peonidin, and petunidin. They differ by position and number of –OH and $–OCH_3$ group present in it. Based on the position of the functional group, either hydroxyl, methyl, sugar moiety, or several other functional substituents, about 500 and more anthocyanins have been identified and are classified under 31 anthocyanidins. Cited previous literature stated that 31 anthocyanidins account for cyanidin (30%), delphinidin (22%), and pelargonidin (18%). Also, other methylated derivatives of anthocyanidins account for 20% of all anthocyanins. Consequently, about 90% of all the anthocyanins are cyanidin, delphinidin, pelargonidin, and its derivatives (methylated). Naturally, these anthocyanidins are present in the form of glycosides with the respective sugar moiety at 3-position on the C-ring or the 5-position on the A-ring to the aglycone chromophores. Basically, pelargonidin can be chemically structured as 1-benzopyrylium, 3,5,7-trihydroxy-2-(4-hydroxyphenyl), chloride, n anthocyanidin cation that is flavylium substituted by a hydroxy group at positions 3′, 5′, 7′, and 4′ (Barnes et al. 2011).

6.3.1 Natural Occurrence of Pelargonidin

Anthocyanins are one among the subgroup of water-soluble flavonoids found widespread in the plant kingdom, and their specific compounds are responsible for floral pigmentation and other parts of plants. The color of the pigments may vary from vibrant red, purple, to blue pigments, and this is because of its conjugated structure that draws light at 500 nm (Wang et al. 2012). In particular, widespread and usually found anthocyanins are the 3-*O*-glycosides or 3,5-di-*O*-glycosides of delphinidin, cyanidin, petunidin, peonidin, malvidin, and pelargonidin. Anthocyanins are important polyphenolic components of fruits, especially berries. Bioactive component

pelargonidin is rich in berries, such as strawberry, blackberry, black currant, elderberry, sour cherry, and pomegranate. Consumption of anthocyanin and polyphenol-rich juice enhanced antioxidant status, reduced oxidative DNA damage, and stimulated immune cell functions (Ferretti et al. 2010).

6.3.2 Pharmacological Activities of Pelargonidin

Flavonoids most prevalently found in floral, fruit, and vegetable have been reported as pelargonidin and consuming them as fresh or processed food has a major impact in prevention and control of devastating chronic human diseases, such as inflammatory diseases, cancer, and cardiovascular diseases, where it directly acts as potent antioxidant, plays significant role in detoxification, induces apoptosis, fights against inflammation, etc. (Nikkhah et al. 2008). The antioxidant property of pelargonidin (anthocyanidin) was due to scavenging efficacy of ONOO− function group, and it was evidently first reported by Tsuda et al. (2003) (anthocyanidin). The mechanism behind its antioxidant activity was due to the conversion of pelargonidin to p-hydroxybenzoic and further formation of 4-hydroxy-3-nitrobenzoic acid by the catalytic conversion with ONOO− present in its structure. In vitro studies on anthocyanin had significantly downregulated the signaling pathways including NF-κB and MAPK pathways, resulting in the reduced proinflammatory cytokine expression and inhibiting inflammation (Wang et al. 1999; Pergola et al. 2006). In addition, extensive study on literature of anthocyanidin unraveled its anti-inflammatory mechanism, where significant downregulation of certain vital inflammatory markers at both transcription and translational levels in some cell line models such as downregulation of cyclooxygenase-2 (COX-2) expression in stimulated macrophage (RAW 264 cells) with lipopolysaccharide (LPS) and another case potential inhibition was observed against the transcription and expression of inducible nitric oxide (iNOS) murine macrophages (J774) activated with LPS (Hou et al. 2005; Hämäläinen et al. 2007). Isolated leucopelargonidin-3-*O*-alpha-L rhamnoside from the bark of *Ficus benghalensis*, oral administration at a dose of 100 mg/kg, showed a significant decrease in glucose level with a rise in serum insulin level of diabetic dogs induced by alloxan at a time period of 2 h. The findings state that leucopelargonidin-3-*O*-alpha-L rhamnoside stimulates insulin secretion (Augusti et al. 1994). Also, few studies have reported the estrogenic property of anthocyanidins, including pelargonidin, delphinidin, and cyanidin. These anthocyanidins were categorized as nongenotoxic, and it efficiently modulates DNA damage caused by oxidative stress and 4-nitroquinoline 1-oxide by its estrogenic activity. Therefore, the stated study has substantial evidence for the chemopreventive effects of anthocyanins against carcinogenesis on HL-60 cells (Abraham et al. 2007). A recent study on *F. benghalensis* root extract showed an antimicrobial activity when compared to the standard drug. Also, two important flavonoid compounds such as leucopelargonidin 3-*O*-alpha-L rhamnoside and 5, 3′-dimethyl ether of leucocyanidin 3-*O*-alpha-D galactosyl cellobioside were isolated from the bark of *F. benghalensis*, which inhibits the Gram-negative bacteria and also the fungal species (Aswar et al. 2008).

6.3.3 Bioavailability

Till now, very few numbers of literature are available to understand bioavailability including absorption/excretion of anthocyanins in humans, still, we found some of the contradictory conclusions. Previous studies have shown that wine from graphs rich in pelargonidin, it may or may not contain alcohol do not block the anthocyanin's absorption (Bub et al. 2001; Frank et al. 2003). Later, few other studies by Wu et al., (2006) also stated the similar pattern of specific anthocyanin pelargonidin-3-*O*-glucoside absorption form strawberries and blackberries (Wu et al. 2006). Felgines et al. (2007) stated that pelargonidin-3-*O*-glucoside present in strawberry was metabolically converted into respective pelargonidin glucuronides and pelargonidin sulfate and got excreted in the urine along with a very minute concentration of pelargonidin glucoside and pelargonidin alone a trace of pelargonidin as parent glucoside and its aglycone. More than 80% of urinary excreted pelargonidin accounts as pelargonidin glucuronides (Felgines et al. 2005). Bioactive compound like Cyanidin-3-*O*-glucoside, 3DA or 3 deoxy derivative, pelargonidin-3-*O*-glucoside, which is present in blackberry was reported as much as less than ten times its bioavailability with the corresponding 0.16% of intake and it is excreted as glucurono, sulfo and methylated metabolites which account for about 13% (Felgines et al. 2005). Pelargonidin glucuronides along with pelargonidin-3-*O*-glucoside are the two forms of anthocyanidins from strawberries found in the blood stream, and it undergoes a metabolic modification before being excreted in the urine along with trace quantities (1–3%) of pelargonidin and pelargonidin-*O*-sulfate, whereas the remaining quantity of pelargonidin was detected in plasma. In human beings on ingestion with strawberries pelargonidin-3-*O*-glucoside, it was metabolically converted to pelargonidin-*O*-glucuronide, which is present predominantly in both plasma and urine rather than the parent glucoside. The Cmax of this metabolite was found to be 274 ± 24 nmol/l after 1.1 ± 0.4 h. The major anthocyanin in strawberries, pelargonidin-*O*-glucuronide, was excreted after its metabolic process within the period of 24 h after its intake (Mullen et al. 2008).

6.4 Anticancer Mechanisms of Pelargonidin

6.4.1 Anticancer Activity Via Inducing Apoptosis

Pelargonidin, an anthocyanin found widespread in most of the berries including blackberry, raspberry, strawberry, and pomegranate, were studied in human colon adenocarcinoma cells (HT-29) (Felgines et al. 2007; Beekwilder et al. 2005). The primary anticancer effect of pelargonidin was screened with well-defined in vitro MTT assay on HT-29 cells. Followed by treatment with a determined concentration of pelargonidin (GI_{50}) against HT-29, induction of apoptosis in cancer cells had been reported. Also, the same study had stated the activation of caspase-dependent apoptotic pathway along with the fragmentation of genomic DNA. Additionally, the microscopical observation of treated cancer cells was found to be pictured with

distinct morphology, including floating cells, cellular shrinkage, and membrane blebbing, which are the hallmark features of apoptosis. A similar pattern of cell morphology in another study was reported as characteristic features of apoptotic induction in cancer cells (Sivalokanathan et al. 2006). HT-29 cell architecture had been pictured with high alterations such as round-up of cancer cells with distorted patterns (Karthi et al. 2016). AO and EtBr (AO/EtBr) fluorescent screening had further pictured the intensive morphology of apoptosis-induced HT-29 cancer cells after pelargonidin treatment in a dose-dependent manner. Basically, AO dye stains both live cells with the rigid cell membrane and permeable dead cells, whereas EtBr stains only apoptotic cells with no cell membrane integrity (Raju et al. 2004). Pelargonidin supplement had reported the notable percentage of both early and late apoptotic colon cancer cells with morphological alterations during apoptosis such as cell shrinkage along with chromatin condensation.

The development and maintenance of a living organism have to be brought by the standard and controlled the homeostatic balance of both cell proliferation and cell death. Numerous physiological functions and pathological processes were based on the key role played by the apoptosis of individual cells. Modulation in the expression pattern of certain antiapoptotic proteins (Bcl-XL, Bcl-2, and A1) was notably unregulated by the specific transcription factor of NF-κB, and that was a key role in the inflammation process. Also, the unique transcription factors of this Bax family along with the contribution of NF-κB induce apoptosis. Apoptotic induction by the pelargonidin in HT-29 cells was illustrated with DNA fragmentation protocol, which has been considered as exemplary biochemical features of apoptosis. The ladder pattern after pelargonidin treatment was evident as a result of oligonucleosomal fragmentation of chromosomal DNA at the early apoptotic stage. The previous research study had stated that pelargonidin with short incubation time brings about early apoptosis and longer exposure of pelargonidin was reported to increase the volume of nuclear fragmentation which evidences the basal mechanism of anticancer effect of the pelargonidin, and also it may act through the apoptotic signaling. Bcl-2 family are known cytoplasmic proteins that regulate apoptosis, in particular sub-group of pro-apoptotic proteins Bax, significantly promotes cell death where other members of the Bcl-2 possess anti-apoptotic activity namely, Bcl-x Land Bcl-w, those includes four regions of similarity with Bcl-2. In this review, it is significant to pinpoint that pelargonidin alters the expression level of Bcl-2 family proteins in cancer cells and thereby induces apoptosis. Consciously, the expression of these particular proteins after pelargonidin treatment was deliberated with the immunoblot analysis, and the study has reported that the expression level of Bax was found to be markedly increased dose dependently in HT-29. At the same time, pelargonidin modulations in BH3 interacting-domain (BID) death were also upregulated. As per the study by Karthi et al., (2016), this treatment also downregulates the expression of antiapoptosis protein Bcl-2 and Bcl-xL Briefly, this review conveys that pelargonidin specifically promotes the translocation of mitochondrial Bax to the cytosol and triggers the signaling pathway for apoptosis in colon cancer cells.

6.4.2 Pelargonidin and Mitochondrial Pathway

The apoptotic proteins from the mitochondria due to its permeabilization make the cancer cells to induce apoptosis by three major toxic proteins: direct caspase activators, indirect caspase activators, and caspase-independent proapoptotic factors. Initially, the apoptosome was formed by conjugation of cytochrome c with apoptosis-protease activating factor pro-caspase-9, thereby caspase-9 gets activated. Subsequently, this initiator caspase-9 activates downstream caspases 3, 6, and 7, which were denoted as effectors, thereby it activates the last step of the pathway to induce apoptosis (Pradelli et al. 2010). Tumor suppressor p53 induces autophagy and/or apoptosis through its cytosolic and nuclear effects in response to DNA damage and other stresses. Oxidative stress, i.e., increased production of reactive oxygen species (ROS), can also induce apoptosis and autophagy (Fimia and Piacentini 2010). As in the case of pelargonidin treatment against colon cancer cells, Bcl-2 family members that come under proapoptotic activity significantly activate mitochondrial-mediated intrinsic apoptosis, where pelargonidin potentially induces the loss of mitochondrial membrane potential, which in turn activates caspase-9 cascade. Briefly, the cascade was initiated with the release of cytochrome C followed by activation of caspase 3 and caspase 7, where the program cell death was initiated with sequential degradation of cellular functional proteins (Karthi et al. 2016; Adams 2003). On the other hand, this review also explores the upregulation of apoptotic proteins on pelargonidin exposure such as PARP and P53. Based on the previous literature and the various study reports, the current review has summarized the activation of the intrinsic apoptotic mechanism of pelargonidin against colon cancer cells.

6.4.3 Pelargonidin Affects Cell Cycle Regulators

Tumor cell possesses unique characteristic futures by modulating the regulation of the cell cycle in cancer cells. This chapter also concentrates on elaborating on the mechanism of cell cycle arrest induced by pelargonidin in colon cancer cells. Basically, the transition between cell cycle phases is tightly regulated by cyclin-dependent kinases (Cdk). The role of Cdks is to control cell cycle progression by phosphorylation of protein substrates on serine and threonine amino acids. The protein substrates have specific roles that contribute to the cellular events that occur during the cell cycle. For example, Lamin B protein is phosphorylated by Cdk1 during mitosis, which causes nuclear envelope break down. The correct timing of cell cycle phase changes is regulated by specific cyclin-dependent protein kinase complexes. Cyclin-dependent kinases are composed of two proteins: a catalytic subunit known as Cdk and a regulatory protein subunit known as cyclin. The cyclin proteins do not have enzymatic activity; however, they bind and activate the catalytic 6 subunits. At least 25 catalytic Cdks have been described, and each binds to at least one of a large family of cyclin binding partners, creating many possibilities for Cdk cyclin protein pairings (Bruyère and Meijer 2013). The timing of the synthesis of specific cyclins is carefully regulated, which ensures that certain Cdk-cyclin

complexes trigger different stages of the cell cycle. For example, the Cdk4/cyclin D complex functions early in the G1 phase of the cell cycle in response to growth factors, whereas the Cdk1/cyclin B complex enables cells to enter mitosis. There are several biochemical steps required to activate Cdks in addition to cyclin synthesis, thus ensuring cell cycle fidelity. The cell is blocked in the G2 phase of the cell cycle if Cdk1 remains phosphorylated on tyrosine 15, which holds the enzyme in an inactive state regardless of cyclin B1 levels. Eukaryotic cells (including human cells) have a second biochemical system that can regulate CDK activity if the cell has damaged DNA. Another protein kinase, Chk1 (Checkpoint kinase 1), can prevent the activation of Cdk1 by phosphorylating and promoting the degradation of Cdc25 phosphatases. Without Cdc25 phosphatases, cells cannot dephosphorylate tyrosine 15 of the catalytic subunit of Cdk1, thus remain blocked in interphase, usually the G2 phase. Overall, a series of protein phosphorylation and protein synthesis pathways converge to regulate cyclin-dependent protein kinases. It appears that the complexity of these steps provides cells with opportunities to ensure that their genomes are accurately copied and distributed to daughter cells while avoiding genome change (Smith et al. 2007). This part of the chapter specifically focused to reveal the cell cycle arrest and checkpoint adaptation due to induced DNA damage and interface with the cancer cells to enter mitosis. Cells with damaged DNA initiate a biochemical pathway called the "DNA damage checkpoint," which causes a delay in the cell cycle during S phase or G2 phase to allow repair. It is believed that the role of DNA damage checkpoints is to prevent damaged DNA from being transmitted to daughter cells. Cells with damaged DNA initiate a biochemical pathway called the "DNA damage checkpoint," which causes a delay in the cell cycle during S phase or G2 phase to allow repair. Thus, the review study has strongly deliberated the role of pelargonidin-induced DNA damage, and its role in cell cycle checkpoints is to prevent damaged DNA from being transmitted to daughter cells. The DNA damage checkpoint is composed of several overlapping checkpoint systems. S-phase checkpoint provides continuous monitoring of the DNA during DNA replication to ensure that blocked replication complexes or damaged DNA were repaired before replication. The successful completion of DNA replication is assessed in the G2 checkpoint. The G2 checkpoint enables cells to detect unreplicated DNA or DNA that may have been damaged by a variety of means such as genotoxic agents. The cell will delay its progression through the cell cycle at the G2 phase until errors are corrected (Palou et al. 2010). The previous study of our laboratory had strongly pinpointed that the G2/M phase arrest of colon cancer cells was significantly induced by pelargonidin treatment in a dose-dependent manner and take part in a potential role of regulating the expression of subsequential cell cycle regulatory proteins (Karthi et al. 2016). Also, pelargonidin has a minimal effect to inhibit the cells at the G1-S phase of the cell cycle in cancer cells and is mainly due to transition-related CDKs, and their expressions were highly modulated by pelargonidin with inhibiting the function of mutated P53 in colon cancer cells. Thus, the literature study on anthocyanidin, pelargonidin the drug of interest, has high efficacy and efficiency to modulate the expression of functional protein in inhibition of CRC, and thereby it possesses a significant anticancer activity which was summarized in this chapter.

6.5 Conclusions

This comprehensive review about the anticarcinogenic effect of pelargonidin concludes that most of the commercial synthetic and isolated compounds used to treat cancer not only kill cancer cells, but are also proven to be highly cytotoxic to normal cells and would cause a severe adverse effect after therapy. Whereas, pelargonidin being pinpoint and speculate that naturally occurring dietary flavonoid acts as an anticancer therapeutic agent, which inhibits colon carcinogenesis by activating the immune system and by regulating the hyperproliferation, inflammation, angiogenesis, and mitochondrial-mediated apoptosis. In particular, this chapter signifies the importance of pelargonidin in the treatment of colon cancer. Briefly, the review study states that pelargonidin can affect the basic cell functions associated with cancer development. Also, it induces significant cell cycle arrest and apoptosis in cancer cells; thereby, pelargonidin would control the formation and progression of tumors. The previous literature evidence that any alteration in the inhibition of proteins involved in the cell cycle would potentially reflect in inhibition of cell proliferation and block carcinogenesis in tumor cells. Thus, this chapter highlights the pharmacological potential of pelargonidin, and it may be very useful in the prevention of oxidative stress-induced diseases and takes part in vanquishing colon carcinogenesis. Further, a detailed study on pelargonidin would effectively promote the development of new antitumor drugs and regimens for human colorectal carcinoma.

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Targeting the Key Signaling Pathways in Breast Cancer Treatment Using Natural Agents

7

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Abstract

Among the many leading types of cancers, breast cancer is placed second, affecting women globally and causing higher morbidity and mortality. Many therapeutic approaches such as chemotherapy, surgery, hormone therapy, and radiotherapy have been employed in the treatments of breast cancer. However, there are several limitations and severe side effects involved. Moreover, the long-term treatments lead to multidrug resistance, owing to which these treatment methodologies have become relatively unsuccessful. In the process of attempting to interpret the basics and biology of cancer and its progression, many significant ways of its prevention, diagnosis, and therapeutics have been discovered recently. Natural plant-derived bioactive compounds have a great deal of cancer prevention abilities. Plant compounds are known to suppress the fierceness of breast cancer by inhibiting cancer cell multiplication and restraining tumor signaling pathways. In this chapter, we have discussed on various phases and risk factors of breast cancer, therapeutic targets, and signaling molecules that are involved in breast cancer. Also, the importance of dietary natural products that have been experimentally proven to prevent breast cancer is detailed in this chapter. Further, some of the major anticancer phytocompounds and their mechanisms of actions are highlighted with experimental evidences. Therefore, this comprehensive information will benefit researchers to understand more about breast cancer and its pathogenesis, and allow one to explore plant-derived bioactive molecules in treating breast cancer.

Keywords

Breast cancer · Signaling · Treatment · Prevention · Natural products

7.1 Introduction

Breast cancer is presently considered to be the most prevailing cancer type among females, and it is the second most prominent mortality causing diseases among women population, globally (Siegel et al. 2018). Breast cancer is ranked the third fatal-most

cancer among the top five cancers in America and is also very much prevalent in the Asian subcontinent (Torre et al. 2015). Survey from population-based cancer registries suggests that in the year 2008 alone, 1,384,155 fresh breast malignance cases have been registered all over the world, and of which nearly 459,000 related deaths have occurred (Tao et al., 2015; Desantis et al. 2014). It has been established that along with the USA and China, India holds one-third of the global breast cancer burden (Ferlay et al. 2015). During the years 2008–2012, in India alone, the number of breast malignancies has risen to 11.54% with 13.82% increased mortality rate (Druesne-Pecollo et al. 2012; Ferlay et al. 2015). A survey by the American Cancer Society suggests that one in every eight females in America is under the risk of developing breast carcinoma during their lifespan. In the USA, there were approximately 252,710 fresh cases of aggressive breast carcinoma and 40,610 demises have been reported in the year 2017 alone. It has also been speculated that the occurrence of breast carcinoma may touch up to 3.2 million fresh cases each year by 2050 (Zohre and Hamid 2019).

Breast cancer has been classified based on the morphology of the cells and evaluation of HER2 (human epidermal growth factor 2) and HRs (hormone receptors) by immunohistochemical analysis. A diverse range of biomarkers have been evaluated to be utilized as prognostic tools. Being a heterogenic disease, breast cancer has the ability to alter the biological system during the course of the disease (Polyak 2011; Sana and Malik 2015). Breast cancer molecular subtypes are as follows: (1) luminal-hormone receptor (HR^+) breast cancer, the most common subtype, and it accounts for nearly 60% of the occurrence. The treatment options include endocrine therapy (ET) in adjuvant and metastatic settings (Cardoso et al. 2018); (2) TNBC (triple-negative breast carcinoma), a subclass of breast cancer that deficit the expression of HER2 and HR. Further, it accounts for nearly 15–20% of breast cancer cases with aggressive nature and risk of relapse. The metastatic TNBC is heterogenic in nature, and regardless of many developments in the field of cancer cure, it still remains as an unresolved medical need with only few therapies being used apart from the standard cytotoxic chemotherapy (Lehmann et al. 2015; Plasilova et al. 2016).

It is known that surgery, radiotherapy, and chemotherapy are the primary treatment options to treat breast cancer. In chemotherapy, the treatment options include

Table 7.1 Ranking and rates for breast cancer

Breast cancer	%[a]	R[b]	CR[c] per 100,000	AAR[d] per 100,000
Mumbai	28.8	1	33.6	33.6
Bangalore	27.5	1	29.3	34.4
Chennai	30.7	1	40.6	37.9
Thiruvananthapuram	28.5	1	43.9	33.7
Dibrugarh	19	1	12.7	13.9
New Delhi	28.6	1	34.8	41
Barshi Rural	20	2	13.2	12.4

[a]Relative proportion
[b]Rank
[c]Crude rate
[d]Age-adjusted rate

certain drugs like anthracycline (e.g., epirubicin, doxorubicin) and taxanes (e.g., docetaxel, paclitaxel) (De Cicco et al. 2018; Akhtar and Swamy 2017). These chemotherapeutic agents show side effects that harm the patients. In order to get rid of the adverse effects of chemotherapeutic drugs, researchers believe that natural agents are the right alternate to treat breast cancer. Previous findings have already proven that certain key natural compounds—curcumin, epigallocatechin gallate (EGCG), shikonin, sulforaphane, and genistein—kill the breast cancer cells (Sarmistha and Raju 2018).

7.2 Breast Cancer Ranking in Indian Population

Breast cancer has ranked top even in the Indian individual registries (New Delhi, Chennai, Bangalore, Mumbai, and Dibrugarh) during 2012–2014 (Malvia et al. 2017). Chennai contributed 30.7% and Dibrugarh had 19% (Table 7.1). The major factors that lead to a rise in breast carcinoma in the urban registries include urbanization and westernization accompanied by varying lifestyle and food habits. However, cervical cancer still holds the top position in females followed by breast cancer in the second position in the Barshi rural registry. Among the registries, Thiruvananthapuram ranked the highest with an average of 43.9 per 100,000 people. Likewise, in Chennai, New Delhi, and Mumbai cities, the crude rate (CR) of breast cancer was found to be 40.6, 34.8, and 33.6, respectively, per 100,000 people. Taking age-adjusted rate (AAR) into consideration, the following cities occupy the top four positions among the Population-Based Cancer Registries in India (PBCRs) with 41.0 per 100,000 in Delhi, 34.4 in Bengaluru, 37.9 in Chennai, and 33.7 in Thiruvananthapuram (Anonymous 2016). Another interesting aspect is the mortality/incidence ratio (MIR), which is used to detect if a region has a greater mortality ratio than its incidence rate. Despite the low incidence of breast cancer, Barshi rural has a high MIR (66.3) exhibiting high mortality rate, whereas the registry in Delhi exhibits MIR as low as 8.0 in spite of the high incidence (28.6%). This is believed to be owing to reasons like greater literacy rates, increased awareness, and reach of better medical facilities in metropolitan cities. The main setback observed in the rural areas is the late diagnosis of cancer—generally in the advanced stages, with a handful of population exhibiting metastasis that is widespread by then. This demands more attention towards creating awareness, providing treatment, and facilitating initial detection of breast carcinoma (Malvia et al. 2017).

7.3 Development of Breast Cancer and Risk Factors

Genetics, hormones, physiology, environment, and sociobiology are a few among the many other interrelated influences that provoke the occurrence of breast carcinoma. Many reports have shown the involvement of various risk factors in breast

cancer development. A list of identified threatening reasons for breast cancer (Ferlay et al. 2015) are as follows:

1. Environmental factors: Exposure to nuclear waste materials that emits ionizing radiation and the use of medical diagnostic equipment or therapeutic procedures that emits radiation has increased the danger of breast cancer incidences.
2. Sociobiological factors: The predominant menace for the breast cancer occurrence includes age and gender. It has been reported worldwide that in women above the age of 50, nearly 75% of fresh incidences with 84% of deaths are reported to occur (Akram et al. 2017). Taking the age factor alone into consideration, women aged 40 and above have a risk aspect ratio of 1 in 232 as compared to those women in their seventh decade of life rated at 1 in 29. The increase in the number of cases may be directly proportional to the hormonal changes occurring in women of this age group (Vishwakarma et al. 2019).
3. Nutritional factors: Consumption of higher amounts of caffeine, red meat, and fat serves as the positive risk factor, causing breast carcinoma. On the contrary, vegetables and fruits lessen the danger of breast carcinoma development. Phytoestrogen, calcium, and vitamin D are few of the many ingredients verified to be effective in plummeting the risks of breast cancer (Desmawati and Sulastri 2019).
4. Physiological factors: Physical activities and regular exercise have proven to moderate breast cancer risks. Some investigations have shown that about 30% reduction in the risk related with breast cancer has been observed while implementing few hours of exercise per week in comparison to no exercise at all (Hardefeldt et al. 2018).
5. Genetic factor: Genetics play a major part in the manifestation of breast carcinoma, where nearly 5–6% of the breast cancer incidence is hereditary. The two major genes responsible for hereditary breast cancer include BRCA-1 and BRCA-2. It has been reported that women who exhibit the expression of BRCA-1 or BRCA-2 are prone to breast cancer risks (up to 50–85%) and ovarian cancer risk (up to 15–65%) (Haber 2002).
6. Alcohol: Researches have proven that consuming moderate level of any type of alcoholic drink has proven to be the causative agent for a number of cancers like breast cancer, oral cancer, esophagus cancer, bowel cancer, liver cancer, laryngeal cancer, pharyngeal cancer, and throat cancer (Greene 2002).
7. Hormonal history: Female hormones are generally used for contraception and menopause treatment, and they are proven to have a role in breast cancer onset. For example, estrogen use in excess quantities may lead to breast cancer. On the contrary, pregnancy and lactation decrease breast cancer risks. Hence, a woman will be at high risk of breast carcinoma, if she has increased menstrual cycle numbers. Also, the risk of getting breast carcinoma is greater in females with late menopause, early menarche, and child bearing after 30 years of age, which means more menstrual cycles and thus more exposure to hormones (Carey 2010).

8. Breast cancer history: Women, having previous breast cancer history (either in the treated one or the other), will have increased probability of its relapse (Shaukat et al. 2013).
9. Obesity: Estrogen levels are generally high in obese women as the fat cells present in their body produce excess estrogen which in turn triggers breast cancer (Ando et al. 2019).
10. Oral contraceptives and hormone replacement therapy: The supplementation of estrogen through oral contraceptives and/or hormone replacement therapies increases the possibilities of breast cancer risks (Thorbjarnardottir et al. 2014).
11. Immune system: A weak immune system can always provoke the development of any type of cancer. These include patients with HIV (human immunodeficiency virus) infection and organ transplant patients who are under medication to downregulate the immunological functions to avoid organ rejections.
12. Infections: In some cases, even viral infections may lead to breast cancers. Cervical cancer in association with human papilloma virus, liver cancer in association with hepatitis B and C virus, and lymphoma in association with Epstein–Barr virus are the best examples (Haber 2002).

The breast cancer symptoms differ among individuals. The most prominent symptoms include swelling or redness in skin, change in shape and/or size of breast(s), fluid leaks from one or both nipples, changes in the appearance of nipples, general breast pain, lumps or nodes inside the breast, extreme tiredness and loosing of weight in a short period, etc. (Waks and Winer 2019).

7.4 Stages of Breast Cancer and Treatment Strategies

Different stages of breast cancer depend on the tumor size (T 1–4), lymph node association (N 1–3), and subsistence of outlying metastases (M 0–1). For stage 0 ductal carcinoma size <0.5 cm diameter, lumpectomy is done; however, for lesions with larger size, adjuvant radiation therapy has been combined with lumpectomy. Mastectomy combined with adjuvant tamoxifen therapy is performed for treating extreme ductal carcinoma, which involves two or more quadrants of the breast (Maughan et al. 2010; Santosh et al. 2018). Stage I and II breast carcinoma individuals have been cured with lumpectomy and radiation therapy, which has the similar results as that of total mastectomy. Overall survival periods of 5–8 years can be achieved through lumpectomy combined with radiation therapy. The systemic chemotherapy along with radiotherapy is used to treat early-stage breast cancers (Akhtar and Swamy 2017; Santosh et al. 2018). On the contrary, tumors of size greater than 1 cm and tumors in the nodes are treated through chemotherapy (Teven et al. 2017).

The locally advanced stages of cancer like stage IIIa and stage IIIc cannot be removed surgically. These resectable types of cancers are treated by radiation therapy combined with modified mastectomy and adjuvant chemotherapy. The size of the primary tumors can be shrunk through neoadjuvant chemotherapy (Chen et al. 2004). Hormone receptor status evaluation is done post adjuvant endocrine therapy.

Multimodal approach is generally followed while treating the stage IIIb, stage IIIc, and inflammatory breast carcinoma. Initially, neoadjuvant chemotherapy is carried out. Once the response is positive towards the treatment, modified mastectomy combined with radiation therapy is done. Chemotherapy post breast-conserving surgery is advised for some patients. To conclude with, there is no cure for the stage IV cancer; however, treatment is intended to increase the rates of survival and improve lifestyle quality. Endocrine treatments are mostly implemented as a first-line treatment for the ER+ (estrogen receptor positive) or PR+ (progesterone receptor positive) cancers having metastases in the bones, soft tissues, or with incomplete and visceral metastases. On the contrary, chemotherapy is highly preferred while treating hormone-resistant breast cancers—ER/PR-negative cancers or cancers exhibiting symptomatic visceral metastases (Carlson et al. 2009).

7.4.1 Local Treatments (Surgery)

It is the procedure in which the cancerous tissues within the breast and its surrounding region are surgically removed without damaging the size of the affected part and other parts of the body. Lumpectomy is defined as a process in which the tumor and the normal tissue around it are surgically removed, and in mastectomy, the affected breasts are entirely removed. Thus, these two are the most commonly implemented local treatment methods (Hack et al. 2015; Santosh et al. 2018).

7.4.2 Radiation Therapy

Radiation therapy employs the utilization of high-energy radiation waves to destroy cancerous cells, which can be applied both internally and externally. Radiotherapy, when applied externally, affects the cancerous tissues. In internally applied radiotherapy (brachytherapy), the radioactive materials are positioned within the body, close to tumors for a particular time period. Radiation therapy is widely applied to avoid the recurrence of cancer and to increase the survival rates at different stages of cancer treatment. Nearly 16% recurrence and 4% death rates are positively influenced when almost 11,000 breast cancer patients in their early stage of cancer were subjected to radiotherapy. Though radiotherapy has proven positive therapeutic effects, they are not without hazardous side effects (Brownlee et al. 2018; Santosh et al. 2018).

7.4.3 Therapeutic Agents for Breast Cancer

There are many therapeutic agents or drugs as they are commonly called, used for breast cancer treatment, and they leave behind adverse drug reactions apart from imparting their original role. These adverse reactions suppress the therapeutic outcome, and it is therefore mandatory to link the pre- and posttreatment aspects with the prevailing treatment regimen (Geay 2013; Santosh et al. 2018). Natural agents

Table 7.2 Therapeutic agents for breast cancer

S. no.	Therapeutic agents or class	Name of the drugs/analog for breast cancer treatment
1.	Alkylating agent	Cyclophosphamide
2.	Antimetabolite	Methotrexate (folic acid analog) 5-fluorouracil and capecitabine (pyrimidine analogs)
3.	Natural product	Vinorelbine (vinca alkaloid) Paclitaxel (taxane) Doxorubicin (antibiotic)
4.	Hormone and antagonist	Tamoxifen (antiestrogen) Letrozole and anastrazole (aromatase inhibitors)
5.	Miscellaneous	Trastuzumab (monoclonal antibody) Lapatinib (protein tyrosine kinase inhibitor)

are one of the classes of curative choices available for breast carcinoma (Khaziae et al. 2017; Pandurangan and Mustafa 2018). A list of such natural agents in Table 7.2 provides detailed information about the different classes of curative drugs applied for treating breast cancer.

7.4.3.1 Endocrine Treatment

Tamoxifen (20 mg tablet) is the benchmark therapy used to treat breast cancer, which is a selective estrogen receptor modulator (SERM) (Riggs and Hartmann 2003). This tamoxifen exhibits different agonist and antagonist features by binding to the estrogen receptors and inhibits the proliferation ability of estrogen. Tamoxifen reduces the risk of recurrence of ER+ breast cancer by 50% and also decreases the morbidity rates up to 28% regardless of lymph node and menopausal status (Smyth and Hudis 2015). However, contradicting to this statement is a study that states that endocrine treatments involving tamoxifen upregulate xenoestrogen's agonistic effects on transmuted estrogen receptors, which are associated with drug resistance and refractoriness (Hess Wilson et al. 2006).

The third-generation aromatase inhibitors like anastrozole, letrozole, and exemestane are extensively applied as an alternative for tamoxifen. It is especially used in postmenopausal ER+ breast cancer women as aromatase is the leading source of estrogen in postmenopausal females, and these agents reversibly inhibit the aromatase enzyme that enables transformation of testosterone to estrogen and androstenedione into estrone. Positive results and better body acceptance than the previous hormonal treatments are observed in postmenopausal females who undergo treatment with these drugs (De Placido et al. 2018).

7.4.3.2 Chemotherapeutic Agents

Few of the common chemotherapeutic routines being implemented in breast cancer treatment are described in this section. Breast cancer metastasis is controlled by a prodrug, cyclophosphamide, which changes into its active form through hepatic intracellular enzymes and eventually affects DNA replication and cell division. Cyclophosphamide has been used as a combination—CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) or with anthracycline as an adjuvant therapy in

treating breast cancer (Waldman and Terzic 2009). Carboplatin and cisplatin are platinum-derived compounds that are being used either as monotherapy or in combination with other treatment regimen to treat breast cancer. Monotherapy using carboplatin has proven to show positive results in nearly 25–35% cases, exhibiting metastatic stage of breast cancer. Platinum compounds are now administered along with gemcitabine and taxanes like drugs (Burch et al. 2005; Perez et al. 2005). Paclitaxel and docetaxel are taxanes, which facilitate mitotic arrest through cellular microtubule element stabilization and are used extensively as either monotherapy or combination regimen. These drugs exhibit slight toxicity; nevertheless, they show excellent results when given on weekly basis during the treatment of breast cancer. Among anthracyclines, doxorubicin and epirubicin are preferred in breast cancer treatment, and several studies elucidate their cytostatic and cytotoxic activities including the generation of free radical, peroxidation of lipids, and direct implications on the membrane. However, the drug's interaction with DNA or DNA-topoisomerase II complex (either covalent bonding or intercalation) and modifications in the bases leading to uncertainties in DNA transcription and replication followed by DNA repair or apoptosis is extensively studied (Eniu et al. 2005; Szuławska and Czyz 2006). Despite these toxic effects, the multidrug combination regimens show positive results in breast cancer treatment. An alternative to taxanes in treating advancing metastatic breast cancer is an oral prodrug, capecitabine, a derivative of fluoropyrimidine, which when acted upon by the enzyme thymidine phosphorylase gets transformed to 5-FU. This resulting 5-FU is as effective as 5-FU given through infusion. Treatment for various cancers like lung cancer, bladder cancer, breast cancer, etc. involves a pyrimidine nucleotide derivative, gemcitabine, which hinders RNA synthesis and DNA replication which has shown to give promising results upon weekly IV infusion. Another drug of equal importance is vinorelbine that effectively treats advanced breast cancer by binding to tubulin and thereby affecting mitotic metaphase (Bates and Eastman 2017).

7.4.3.3 Monoclonal Antibodies and Protein Tyrosine Kinase Inhibitor

When talking about monoclonal antibodies as targeted drugs for treating breast cancer, trastuzumab has proven to increase the rates of survival in patients with breast cancer exhibiting HER2/neu (Blackwell et al. 2010). Several studies state that trastuzumab targets the extracellular domain IV of the receptor HER2 and is clinically safe and works effectively along with vinorelbine, paclitaxel, gemcitabine, or carboplatin when administered 3 weeks once (Romond et al. 2005; Vu and Claret 2012). In 2007, the US-FDA (The Food and Drug Administration) approved a dual inhibitor EGFR and HER2 tyrosine kinases—lapatinib, which was used along with capecitabine to treat HER2-positive breast cancer patients who showed progression even after being treated with trastuzumab and letrozole (combination therapy) against HER2-positive metastatic breast cancer (Toi et al. 2009).

Palliation is the best option for incurable metastasis, but endocrine therapy is employed for treating cancer patients who are positive for hormone receptors, where there is a lack of involvement of visceral organs. However, taxanes and anthracyclines (monotherapy) are prescribed when there is failure in the first-line or

second-line treatments along with widespread visceral involvement leading to advancements in disease progression due to antihormonal treatment or in breast cancers lacking hormone receptors, because toxicities arising from a combination regimen seek prompt medical advice. In short, if former chemotherapies fail, the "risk:benefit" ratio of a new therapy employed must be taken into consideration before implementing any subsequent-line therapies for treating metastatic breast cancers (Westphal et al. 2018).

7.4.4 Therapeutic Targets and Signaling Molecules in Breast Cancer

7.4.4.1 Targeting Ras/MAPK Pathway Members

Ras is a signal transduction effector molecule which functions as a second messenger in triggering intracellular signaling pathways and is activated through mutations in oncogenes in approximately 50% of breast cancers. H-Ras, K-Ras, M-Ras, and N-Ras are components of the Ras protein family which basically are small GTPases triggered by several RTKs (receptor tyrosine kinases) (Giltnane and Balko 2014). HER2 and IGF-1 are two receptors of growth factors that activate Ras proteins, which in turn activate downstream pathways like PI3K/AKT and MAPK through Raf, ERK1/2 and MEK enabling the survival and progression of breast cancer cells. Hence, the best approach for targeting Ras/MAPK pathway is by inhibiting the enzyme farnesyltransferase, which is engaged in the Ras protein's posttranslational modification process. Some of the best and commonly used farnesyl transferase inhibitors (FTIs) drugs are lonafarnib, gliotoxin, and tipifarnib (13% overall clinical benefit rate) (Moorthy et al. 2013). Studies reveal that up to 28% minimization in metastasis-free survival was observed upon sorafenib tosylate and vemurafenib (BRAF inhibitors) and binimetinib and selumetinib (MEK inhibitors) administration, where BRAF and MEK are agents that block the activation of the Ras pathway (Jokinen and Koivunen 2015; Zaman et al. 2015).

7.4.4.2 Targeting PI3K/Akt/mTOR Pathway

The most active pathway in breast cancer is the phosphoinositide 3-kinase (PI3K)/Akt/mammalian Target of Rapamycin (mTOR) (PAM) signaling pathway, which is found to be engaged in cellular proliferation, angiogenesis, differentiation, apoptosis, survival, and longevity. In general, HER2-positive, luminal, and triple-negative breast cancers (TNBC) are triggered by the activation of mutations like PIK3CA, AKT1, FGFR1, loss of PTEN's functional mutations, and overexpression/amplification of several components of the PAM signaling pathway like PDK1, IGF1R (Lauring et al. 2013). Inhibitors of the PAM pathway include drugs like letrozole, buparlisib (PI3K inhibitors) exhibiting a clinical benefit rate of 58.6% and an increased progression-free survival rate by 2 months (Di Leo et al. 2018). Deaths by breast cancer have marginally reduced by 16% when uprosertib and ipatasertib (Akt inhibitors) and rapamycin and its derivatives (mTOR inhibitors) are administered (Lee et al. 2015a, b; Rotundo et al. 2016).

7.4.4.3 Notch Signaling Pathway

Among the most excessively conserved signaling pathways is the Notch pathway that can influence cell-to-cell communication, alter key cellular processes, and regulate the development and progression of breast cancer (Al-Hussaini et al. 2011). Researchers have identified five Notch ligands and four Notch receptors, whose interaction between cells activates the Notch signaling pathway post which cleavage by proteolytic enzymes at an extracellular site occurs resulting in the synthesis of Notch extracellular truncation (NEXT). γ-secretase enables Notch intracellular domain (ICD) formation and its translocation from the cytoplasm into the nucleus, which is the site for it to bind with ubiquitous transcription factor CSL-CBF1 (C-promoter binding factor 1), a protein which binds with DNA and ultimately transforms it into a transcriptional activator and thereby triggers the initiation of diverse downstream pathways (Shih and Wang 2007). Phase II clinical trials are underway for aspartyl protease inhibitors and γ-secretase inhibitors (RO-4929097) in treating TNBC with relapsing property as they efficiently prohibit the entry of Notch ICD's entry into the nucleus, eventually altering the notch signaling pathway. Similarly, for treating stage I and II TNBC, carboplatin, paclitaxel, and RO-4929097 are used in combination, which is under phase I clinical trials (Olsauskas-Kuprys et al. 2013). The Notch ICD when acted upon by γ-secretase dissociates it into two distinct proteins, namely, presenilin (catalytic activity) and nicastrin (helps in gene maturation). When they translocate into the nucleus, they interact with CSL (transcriptional activator) and initiate the transcription of subsequent downstream factors, VEGFR3, ER, HES and HEY, NF-κB2, c-Myc, cyclin D1 and p21, HER2, angiogenic, and apoptotic regulators. In conclusion, promising results in treating TNBC cases can be achieved when γ-secretase inhibitors are used against the Notch signaling pathway. Below is a figure that highlights the activation process of Notch signaling pathway, and its predominant targets for drug design are also shown (Notch-specific) (Fig. 7.1).

7.4.4.4 Wnt/β-Catenin Pathway

Similar to the Notch pathway, yet another promising focus for TNBC therapy is the Wnt/β-catenin signaling pathway, which upon aberrant activation can influence both embryonic and cancerous growths. In many cancers including TNBC, the Wnt/β-catenin pathway is found to be aberrantly upregulated, especially the Wnt co-receptor—low-density-related protein-6 (LRP6) and the Wnt receptor frizzled-7 (FZD7) (Barker and Clevers 2006; Bayet-Robert et al. 2010). Research findings account for the activation of the Wnt/β-catenin signaling in TNBC, in which the Wnt co-receptor LRP6 and the Wnt receptor frizzled-7 (FZD7) were found to be upregulated specifically (King et al. 2012). When cytosolic β-catenin is stabilized, it translocates inside the nucleus and stimulates the Wnt-targeted genes by binding to T-cell factor/lymphoid enhancing factor (TCF/LEF) family (Lu et al. 2011). A supramolecular complex comprising axin, Adenomatous Polyposis Coli (APC), and glycogen synthase kinase 3β (GSK3β) stabilizes the β-catenin levels when Wnt ligands are absent. This leads to the sequential phosphorylation of β-catenin at the amino-terminal region, and this phosphorylated form of β-catenin becomes

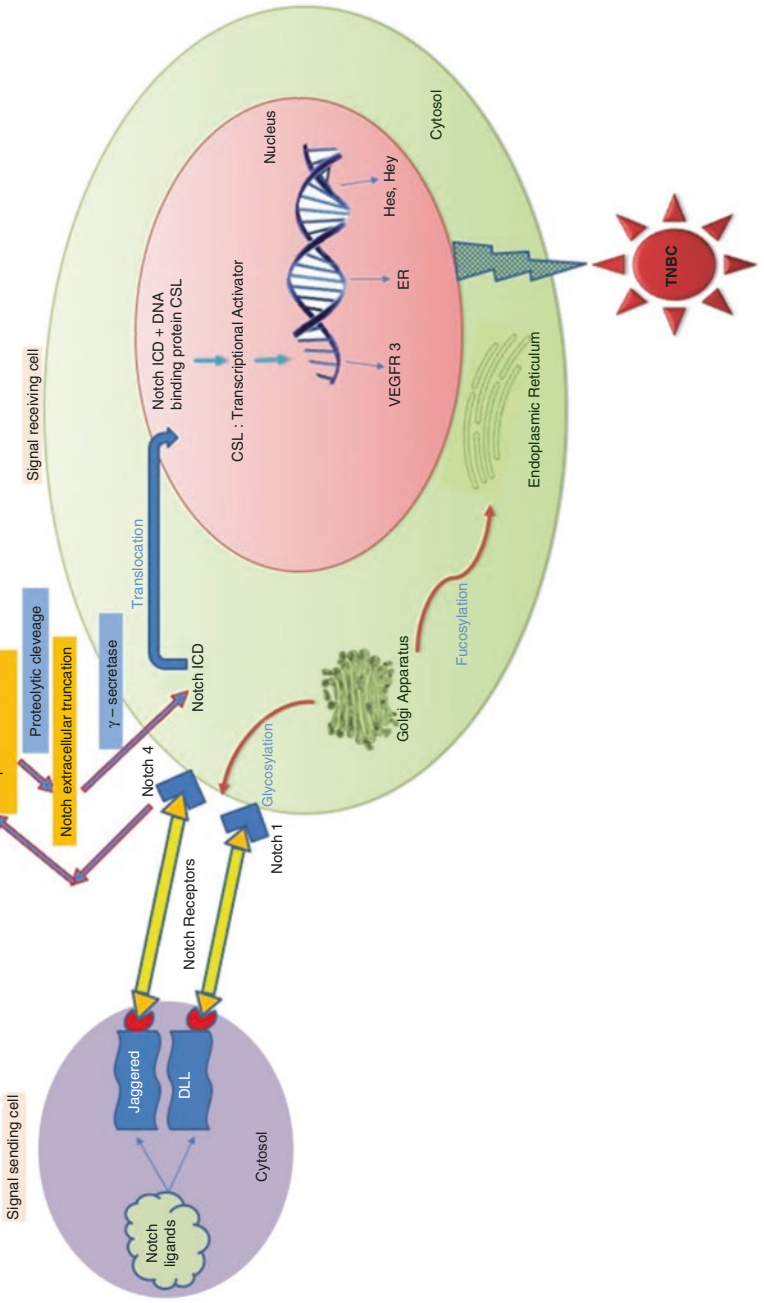

Fig. 7.1 Regulation of notch signaling pathway in breast cancer

multi-ubiquitinated (Ub), which is later acted upon by the 26S proteasome. Thus, the interaction of a Wnt ligand with its receptor on the surface of the cell upregulates the Wnt/β-catenin target genes leading to cancer initiation and progression through uncontrolled control cell proliferation and apoptosis (Barker and Clevers 2006; Bayet-Robert et al. 2010). LRP6 is therefore identified as a vital co-receptor in the Wnt/β-catenin pathway and hence a prospective therapeutic target in treating breast cancers. Recent research on the anticancer properties of Gigantol (medicinal compound isolated from medicinal orchids) in treating breast cancers has proved that it remarkably minimized cytosolic β-catenin, total LRP6, and phosphorylated LRP6 levels, leading to low-level expressions of Axin2 and Survivin (Wnt target genes). LRP6 is degraded when Wnt/β-catenin inhibitory drugs like salinomycin (anticoccidial) and nigericin are administered, which acts on breast cancer stem cells as well (phase I/II clinical study) (Naujokat and Steinhart 2012; Yu et al. 2018).

7.4.4.5 PARP Enzyme Inhibitors

Excision repair of breaks in single-stranded DNA is a process involving PARP1 (Poly ADP-ribose polymerase), which corrects the damaged DNA sequences by repairing base excisions and nucleotide excisions and mismatches by filling the gaps with complementary DNA strand (Veuger et al. 2004). However, in type 1 breast cancer, which does not involve PARP1 during replication, two proteins—BRAC1 and BRAC2—are engaged in a repair mechanism that is error free (homologous recombination) of double-stranded DNA (dsDNA) breaks, accumulated as a result of the replication fork's halting (Godon et al. 2008). Hence, PARP1 inhibitors are promising therapeutic agents for treating TNBC (mutated BRAC genes) owing to their specificity and nontoxicity towards the surrounding healthy cells, which is lacking in the conventional chemotherapies causing secondary cancer formations (Bryant et al. 2005; Farmer et al. 2005; Hwang et al. 2019).

7.4.4.6 Targets for EGFR

Epidermal growth factor receptor (EGFR), an integral part of the HER/erythroblastosis virus oncogene B (ErbB) family, is a transmembrane tyrosine kinase receptor exhibiting various roles in different signaling pathways like producing Akt (PKB) and mitogen-activated protein kinase (MAPK) subsequently inducing drug resistance, regulation of cell proliferation, differentiation, apoptosis, invasion, and angiogenesis (Tabach et al. 2005; Zhang et al. 2007). Various immunohistochemistry and gene expressions studies suggest that EGFRs are abundantly expressed in most cancers and nearly 40–60% of TNBC cases showed an 18% upregulation subsequently (Sarrio et al. 2008; Gluz et al. 2009). Hence, researchers began studies targeting EGFR as a potential therapeutic agent against TNBC (Ueno and Zhang 2011). One such potent drug is Gefitinib (EGFR inhibitor) with 59% oral bioavailability and 6–49 hrs half-life period that is under clinical trial (phase II) against EGFR-positive (metastatic) breast cancer and TNBC. Recent reports reveal synergistic anticancer activities in TNBC cell lines when dasatinib was administered along with cisplatin and cetuximab (anti-EGFR monoclonal antibody). A combination of the above three drugs lead to enhanced inhibition of MAPK and EGFR

phosphorylation along with conspicuous triggering in the apoptosis levels. Dasatinib when administered alone or in combination markedly diminished invasion and migration of the cancer cells in TNBC cell lines. Hence, further studies on dasatinib in combination with other drugs may result in potential outcomes in clinical studies in TNBC patients (Tong et al. 2018).

7.4.4.7 mTOR Inhibitors

The FK506-binding protein, 12-rapamycin-associated protein 1 (FRAP1) with the familiar name mTOR (Moore et al. 1996) is a part of the PI3K family of proteins regulating transcription, survival, cell growth, motility, progression, and protein synthesis (Hay and Sonenberg 2004; Beevers et al. 2006). The mTOR pathway, which is found to be frequently dysfunctional in many human disease conditions—breast cancer included, consists of two specific complexes—mTORC1 and mTORC2, promoting protein synthesis, growth, proliferation, angiogenesis, and metastasis by the induction of a S-phase kinase association protein. Akt transfers signals like existence of energy, nutrients, or growth factors for cell growth (under favorable conditions) or catabolic processes (during stress) to mTORC1, which leads to the suppression of TSC2 and initiation of mTORC1. But, when the levels of ATP are low, TSC2 is activated (AMPK-dependent). mTORC1 signals are reduced through raptor phosphorylation, and the availability of amino acids is signaled to mTORC1 through the Rag and Ragulator (LAMTOR1–3) proteins. mTORC1 in its activated state translates mRNA by phosphorylating 4E-BP1 and p70 S6 kinase (downstream targets), facilitating biogenesis of the ribosomes, autophagy suppression—Atg13 & ULK1, and transcriptional initiation ultimately leading to adipogenesis (mitochondrial metabolism). mTORC2, which activates PKCα, governs the dynamics of the cytoskeleton and also maintains the transport and growth of ions with the help of SGK1. Therefore, combination therapy associating mTOR inhibitors is under clinical trials for treating TNBC. Examples include lapatinib, everolimus, and capecitabine; when given in combination, it was well tolerated and resulted in a 27% response rate seen in the breast cancer patients with heavy penetration and brain metastases in 12-week period. However, further studies in greater detail are necessary to potentially utilize this regimen (Hurvitz et al. 2018).

7.4.4.8 Transcription Growth Factor-β (TGF-β) Signaling Pathway Inhibitors

A key component of the cytokine superfamily TGF-β engaged in embryonic cell proliferation, differentiation, apoptosis, homeostasis, and also selecting cellular functions in adult organs is a 25-KDa protein TGF-β1 that was first identified in human platelets, which performs a crucial part in the healing of wounds (Ghadami et al. 2000), regulating the immune system and inhibiting the secretion and functions of cytokines, namely TNF-α, IFN-γ, and IL-2 (Tiemessen et al. 2003). On the contrary, in myeloid cells, TGF-β1 enhances the expression and secretion of monocytic cytokines—TNF-α, IL-1α, and IL-1β (Wahl et al. 2006). The emergence of tumor-initiating cancer stem cells (CSCs) has been disclosed among diverse types of cancers, breast cancer included and reports suggest that TGFβ-mediated EMT

(epithelial–mesenchymal transition) cell transformations are associated with BCSC (breast cancer stem cell) formation during epithelial tumorigenesis and provide inflammatory signaling responses in order to sustain their stem-like properties, and these CSCs, possessing the capability to differentiate and self-renew in order to maintain heterogeneity in huge tumor populations and also to provide resistance against chemotherapeutic agents, are being used as therapeutic targets (Woosley et al. 2019). The different pathways and targets of the development of TNBC are shown in Fig. 7.2.

TGF-β inhibitors inhibit the progression of chemotherapeutically resistant tumor inducing cells (TIC) in vivo, and hence their role in combinational chemotherapy for treating TNBC patients is being studied (Bhola et al. 2013). TGF-β induces tumor-like properties within the mammary cells through epithelial-to-mesenchymal transition (EMT), which can be reverted with the help of TGFBR1/2 inhibitors eventually leading to mesenchymal-to-epithelial differentiation within the mammary epithelial cells (Shipitsin et al. 2007; Bhola et al. 2013). In the tumor microenvironment of TNBC, which comprises either tumor cells or immune and stromal cells associated with tumor, the level of TGF-β ligands is found to be increased (Wahl et al. 2006). The SMAD2/3 and SMAD4 produced from the TGF-β pathway are engaged in processes like synthesis of proteins, growth and metastasis of tumor cells, and angiogenesis, thus proving TGF-β pathway's contribution towards development of breast carcinomas and eventually enabling the use of TGF-β inhibitors as antimetastatic drugs in treating cancers (Chen et al. 2019).

7.4.4.9 STAT3 as Target for TNBC Patients

Seven highly functionally and structurally similar associates—STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 of the Signal Transducer and Activator of Transcription (STAT) family of proteins—constitute a DNA-binding domain (DBD), an amino acid domain (NH2), a C-terminal transactivation domain (TAD), a linker domain, a coiled-coil domain (CCD) for interacting with proteins, and a SRC homology 2 (SH2) domain enabling phosphorylation and dimerization (Furtek et al. 2016). Of the seven, preclinical and clinical evidences state that STAT3 has a crucial part in TNBC, and their suppressors effectively diminish the growth rate and metastasis of tumors. STAT3's oncogenic potential has been proved by its ability to express genes involved in processes like invasion, cell proliferation, migration, immune suppression, antiapoptosis, chemoresistance, angiogenesis, autophagy, and maintenance and self-renewal of stem cells (Guanizo et al. 2018; Yu et al. 2014). In TNBC, overexpression of STAT3 is observed, which is linked with tumor induction, proliferation, metastasis, chemotherapeutic resistance, and low survival rates. STAT3 also enhances the expression of cancer-related genes and increases physical interactions and functions of various transcription factors (Sirkisoon et al. 2018).

In TNBC, STAT3 enhances the levels of cyclin D1, survivin, c-Myc, B-cell lymphoma-2 (Bcl-2), and B-cell lymphoma-extra-large (Bcl-xL), leading to cell progression and hindered apoptosis. In TNBC, the transcriptional activation by STAT3 binding to survivin promoter is obstructed when CBP-mediated STAT3 acetylation and nuclear export factor exportin 1 (XPO1) inhibition take place

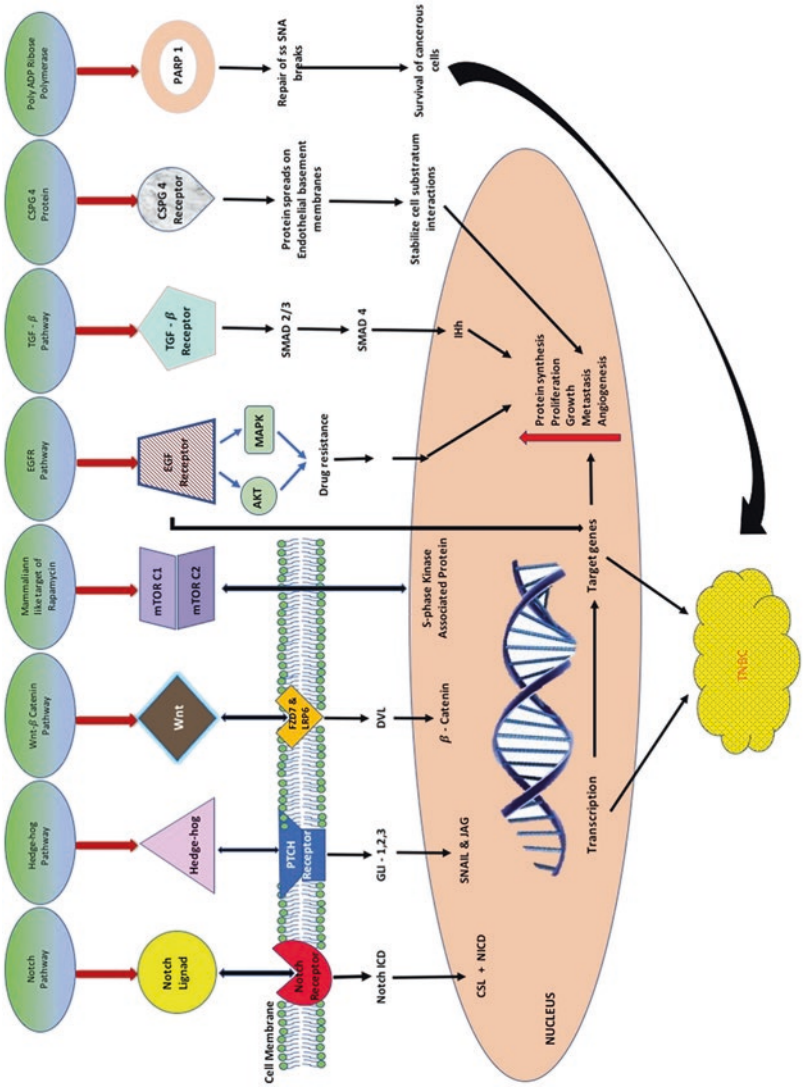

Fig. 7.2 Signaling pathways involved in the development of TNBC acts as targets for the therapeutic approach

Table 7.3 STAT3 inhibitors and their mechanisms of action for TNBC therapy

Name of inhibitors	Mechanisms of action	In vitro activity	In vivo activity	References
1. Target upstream regulators of STAT3				
Carfilzomib	Inhibits IL-6/STAT3 signaling pathway	Inhibits mitosis and proliferation and induces apoptosis	Reduces serum IL-6 levels in tumor-bearing mice	Vyas et al. (2017)
Bazedoxifene	Inhibits IL6/gp130/STAT3 signaling pathway	Inhibits cell viability, colony formation, and cell migration and synergistically enhances the activity of paclitaxel	Suppresses tumor growth	Tian et al. (2019), Fu et al. (2019)
Deguelin	Inhibits EGFR/STAT3 signaling pathway	Inhibits cell viability	Suppresses tumor growth	Mehta et al. (2013)
Ganoderma lucidum extract	Inhibits IL-6/JAK/STAT3 signaling pathway	Inhibits cell viability and induces apoptosis	Suppresses tumor growth	Rios-Fuller et al. (2018)
Nintedanib	Modulates SHP-1/p--STAT3 signaling pathway	Inhibits cell viability and induces apoptosis	Suppresses tumor growth	Liu et al. (2017)
Isolinderalactone	Enhances SOCS3-mediated STAT3 dephosphorylation	Inhibits cell viability/colony formation and induces apoptosis	Suppresses tumor growth	Yen et al. (2016)
2. Directly bind to STAT3 and inhibit its activation				
Bt354	Directly binds to SH2 domain of STAT3 and inhibits phosphorylation	Inhibits cell viability, induces G2/M phase arrest and apoptosis, and impairs cell migration	Suppresses tumor growth	Chen et al. (2018)
Osthole	Directly binds to STAT3 and inhibits its phosphorylation	Inhibits cell viability and induces G2/M phase arrest and apoptosis	Suppresses tumor growth	Dai et al. (2018a, b)
Arctigenin	Directly binds to SH2 domain of STAT3 and inhibits its phosphorylation and DNA-binding ability	Inhibits cell viability, induces apoptosis, impairs cell migration and invasion, and sensitizes cells to chemotherapy	Suppresses tumor growth	Feng et al. (2017)

(continued)

Table 7.3 (continued)

Name of inhibitors	Mechanisms of action	In vitro activity	In vivo activity	References
Alantolactone	Directly binds to SH2 domain of STAT3 and inhibits its phosphorylation	Inhibits cell viability and colony formation and impairs cell migration and invasion	Suppresses tumor growth	Chun et al. (2015)
Strategy 3: Inhibit STAT3 phosphorylation or acetylation				
Sesquiterpene lactones fraction of *Inula helenium L*	Inhibits STAT3 phosphorylation and nuclear translocation	Inhibits cell viability and induces apoptosis	Suppresses tumor growth	Chun et al. (2018)
Rhus coriaria	Inhibits STAT3 phosphorylation	Inhibits angiogenesis and impairs cell migration and invasion	Suppresses tumor growth and metastasis	El Hasasna et al. (2016)
Schisandrin B	Inhibits STAT3 phosphorylation and nuclear translocation	Inhibits cell viability, colony formation, induces cell cycle arrest and apoptosis, and impairs cell migration	Suppresses tumor growth	Dai et al. (2018a, b)
Niclosamide	Inhibits STAT3 phosphorylation and nuclear translocation	Reverses acquired radioresistance	Sensitizes tumors to irradiation	Lu et al. (2018)
Flubendazole	Inhibits STAT3 phosphorylation	Inhibits cell viability, induces G2/M phase arrest and apoptosis, and suppresses BCSC-like phenotype	Suppresses tumor growth, angiogenesis, and metastasis	Oh et al. (2018)
Disulfiram	Inhibits STAT3 expression and phosphorylation	Inhibits cell viability, induces apoptosis, and impairs cancer stem cell-like properties	Suppresses tumor growth and BCSC-like properties	Kim et al. (2017)
SH-I-14	Inhibits STAT3 acetylation and disrupts DNMT1-STAT3 interaction	Inhibits cell viability	Suppresses tumor growth	Kang et al. (2017)

(Cheng et al. 2014; Wang et al. 2018). Progression of tumor in TNBC is also due to the binding of integrin β1 with a β-galactoside-binding protein—Galectin-1, which leads to the initiation of the integrin β1/FAK/c-Src/ERK/STAT3/survivin pathway. TNBC proliferation is suppressed when WW domain-containing oxidoreductase (Wwox) connects with Janus kinase (JAK2), thereby suppressing the phosphorylation of JAK2 and STAT3 (Nam et al. 2017; Chang et al. 2018). Similarly, the levels of IL-6 are reduced considerably when Wwox hinders the interaction between STAT3 and IL-6 promoter. Apoptosis is induced in an ER expression-free manner with the help of gametogenetin-binding protein 2 (GGNBP2)—a tumor suppressor gene that effectively stalls the progression of breast cancer cells and hence adverse effects on the metastasis and growth of TNBC cells can be achieved through suppression of IL-6/STAT3 expression by GGNBP2 (Liu et al. 2019), thus emphasizing that STAT3 is a positive molecular target against TNBC. Table 7.3 lists the various STAT3 inhibitors proposed for TNBC models in vitro and in vivo, Src homology 2 (SH2), glycoprotein 130 (gp130), Src homology region 2 domain-containing phosphatase-1 (SHP-1), DNA methyltransferase 1 (DNMT1), breast cancer stem cells (BCSC), IL6 (interlukin6), and suppressor of cytokine signaling 3 (SOCS3).

7.4.4.10 Noncoding RNAs (NcRNA) as Potential Therapeutic Targets in Breast Cancer

Long noncoding RNAs (NcRNAs) indulged in biological processes like regulation of gene transcription and posttranscriptional and epigenetic modifications are approximately 200 nucleotides fragments of a large group of RNA transcripts (Fang and Fullwood 2016). Recently, they are being extensively studied as alternatives in treating breast cancers owing to the fact that they are potent regulators of gene expression. Researches have proved the existence of two types of NcRNAs—small and long, and both the classes differentially express themselves in different developmental stages of cancer (Hu et al. 2014; Yuan et al. 2014). The latest disease monitoring and individualized treatment regimens used as conventional methods for diagnosis and prognosis of metastatic breast cancer revolve around the use of new and noninvasive biomarkers, and the next-generation sequencing reveals how aberrant NcRNA expressions and different types of cancers are related (Kashi et al. 2016). HOX transcript antisense intergenic RNA (HOTAIR), zinc finger antisense 1 (ZFAS1), H19, TGF-β-activated lncRNA (lnc-ATB), ASBEL, growth arrest-specific gene 5 (GAS5), BC200, X-inactive-specific transcript (XIST), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), SRA1, LSINCT5, and an antisense noncoding RNA in the INK4 locus (ANRIL) are the pivotally deregulated NcRNAs in breast cancer. Of these, the H19 upregulates the tumorigenic properties and triggers paclitaxel resistance (PTX) in ERα-positive breast cancer cells by stalling two proapoptotic factors—BIK and NOXA (Si et al. 2016). However, the long noncoding RNA (lncRNA) inhibitor technology is still in the budding stage, and hence detailed studies on its structure, folding, interaction and functions related to toxic effects, off-course effects, and mode of delivery are underway.

7.4.4.11 Targeting MicroRNAs

Small noncoding RNA transcripts of length 18–23 nucleotides fall under the MicroRNAs (miRNA) category which effectively regulates gene expression by targeting messenger RNA, leading to translational repression or degradation of RNA. More than 4000 miRNAs have been identified that can regulate up to 30% of the entire human genes. miRNAs are aberrantly regulated in different cancers which are linked to the different stages of the disease development—tumor initiation, progression, metastasis, and drug resistance (Mondanizadeh et al. 2015). miRNAs act as both tumor suppressors and tumor promoters; however, their dysregulation is associated with cellular processes of the metastatic cascade, such as sustained proliferation, angiogenesis, and epithelial–mesenchymal transition (EMT) (Zhou et al. 2015). The circulating miRNAs in breast cancers are very stable and can be easily quantified by real-time quantitative reverse transcriptase PCR; hence, they are extensively used in the prognosis, diagnosis, and in studying the response to treatment against drugs (Casey et al. 2016; McAnena et al. 2018). In breast cancers, miRNA functions as either oncogenes/tumor initiators (oncomiRs) or suppressors of tumors, and recent reports reveal the presence of at least one target gene per miRNA (confirmed through experiments). Though miRNA-based therapies are lucrative, few points must be taken into consideration prior to implementation: (1) enhancing the efficient delivery (in vivo) of miRNAs and anti-miRNAs by excluding its deterioration and stability issues, (2) targeting their specificity and minimizing unnecessary aftereffects, (3) re-modeling its design to lower its toxic and off-target effects, and (4) employing engineered models (in vivo) to study its role in the cancer therapeutics (Price and Chen 2014; Christopher et al. 2016). A list of well-documented miRNAs and their target genes related to breast cancer angiogenesis are shown in Table 7.4.

7.5 Limitation and Future Perspectives of Breast Cancer Treatment

When breast cancer treatment is taken into consideration, the main setbacks can be rectified by improving the accuracy and flexibility and implementing cost-effective measures of the drugs. Though targeted therapies like small molecule inhibitors and monoclonal antibodies as combination regimens with conventional chemotherapy are already in practice, there are two major limitations to it—incomplete target inhibition and high rates of harmful side effects and toxicities. Recent progress in research has enabled the use of noncoding RNAs like lncRNAs and miRNAs in both diagnostic and therapeutic segments, and when they are used in combination with chemotherapy, they reduce the resistance to drugs, reduce adverse side effects, and increase target efficiencies. However, the complete knowledge on the deregulation of lncRNAs and miRNAs and its part in the furtherance of breast cancer along with a little development in their off-target effects and delivery efficiency will be an undisputable asset to the patients undergoing breast cancer treatments (Malih et al. 2016; Klinge 2018).

Table 7.4 miRNAs and their target genes associated with breast cancer angiogenesis

S. no.	miRNA	Target genes[a]	Role in breast tumor angiogenesis	References
1	miR-126	VEGFA and PIK3R2	Decreases VEGF/PI3K/AKT signaling activity	Zhu et al. (2011)
2	miR-98	ALK4 and MMP11	Inhibits tumor angiogenesis and invasion	Siragam et al. (2012)
3	miR-148a/152	DNMT1, IGF-IR, and IRS1	Suppresses tumorigenesis by targeting IGF-IR and IRS1	Xu et al. (2013)
4	miR-519c	HIF-1α	Suppresses hypoxia-inducible factor-1α expression and tumor angiogenesis	Cha et al. (2010)
5	miR-10b and miR-196b	HOXD10	Regulates EPC function and tumor angiogenesis through modulation of the Hox pathway	Plummer et al. (2013)
6	miR-216a, miR-330, and miR-608	CD44 3′-UTR and CDC42 3′-UTR	Induces apoptosis, promotes endothelial-cell activities, favors angiogenesis, and increases sensitivity to chemotherapeutics	Jeyapalan et al. (2011)
7	miR-494	PTEN	Activation of the Akt, NF-κB, and mTOR pathways and induction of MMPs expression important for tumor progression	Liu et al. (2012)
8	miR-19	Tissue factor	Inhibits tumor angiogenesis and metastasis	Zhang et al. (2011)
9	miR-145	N-RAS and VEGF-A	Inhibits tumor angiogenesis, cell growth, invasion, and tumor growth	Zou et al. (2012)
10	miR-155	VHL	Promotes angiogenesis, proliferation, tumor necrosis, and proinflammatory cells such as TAMs recruitment	Kong et al. (2014)
11	miR-17-92 cluster	HIF-1α	Impairs cellular adaptation to hypoxia including angiogenesis	Taguchi et al. (2008)
12	miR-140-5p	VEGFA	Inhibits metastasis and angiogenesis	Lu et al. (2017)
13	miR-204	ANGPT1 and TGFβR2	Suppresses vascularization and angiogenesis in breast cancer in vivo models	Flores-Pérez et al. (2016)
14	miR-467	TSP-1	Antagonists of miR-467 and prevents hyperglycemia-induced breast tumor angiogenesis	Bhattacharyya et al. (2012), Krukovets et al. (2015)
15	miR-206	VEGF, MAPK3, and SOX9	Inhibits TNBC breast cell invasion and angiogenesis	Liang et al. (2016)

[a]Activin-like kinase (ALK) 4; Matrix metallopeptidase 11 (MMP 11); Insulin-like growth factor 1 (IGF-1); Insulin receptor substrate 1 (IRS-1); Hypoxia-inducible factor 1-alpha (HIF-1α); Homeobox D10 (HOXD10); Cell surface adhesion receptor (CD44); Phosphatase and tensin

(continued)

Table 7.4 (continued)

homolog (PTEN); N-RAS (N-ras proto-oncogene); Von Hippel–Lindau disease (VHL); Angiopoietin 1 (ANGPT1); Transforming growth factor, beta receptor II (TGFβR2); Thrombospondin-1 (TSP1); Vascular endothelial growth factor (VEGF); mitogen-activated protein kinase 3 (MAPK-3); Sex-determining region Y-box9 (SOX9)

Immunotherapy in recent days is the widely preferred therapy to treat breast cancer, which is under clinical trials and is showing efficient results. Examples include FDA-approved inhibitors of immune checkpoints like the anti-programmed death-ligand 1 (PD-1/PD-L1) and anti-Cytotoxic T lymphocyte-associated antigen (CTLA-4 mAbs), which exhibit strong clinical outcomes in patients with breast cancer exhibiting poor responses. Similarly, vaccines that are cell and tumor-related antigen based which exhibited positive results in clinical trials are yet to receive FDA approval for treating breast cancers. An even more advanced technology is genome editing using "Clustered Regularly Interspaced Short Palindromic Repeats" (CRISPR-Cas9) through which better treatment options can be delivered. However, certain limitations like off-target effects and low delivery efficiencies when resolved may pave paths to cure breast cancer completely.

7.6 Dietary Natural Products for Prevention and Treatment of Breast Cancer

In general, when fruits and vegetables of all kinds are consumed, the risk of many diseases owing to different unhealthy life styles is considerably reduced. The best preventive strategy for cancer lies in proper diet and complete nutrition, which has shown to both prevent and treat cancers. The recent year's publications of meta-analysis report including 93 studies revealed the fact that a wholesome and healthy food pattern followed by postmenopausal and hormone receptor–negative women can effectively bring down the liability of breast cancer (Zheng et al. 2016; Zhou et al. 2016). A diet plan inclusive of soy products, fruits, and cruciferous vegetables has prominently reduced the incidence of breast cancer according to several epidemiological studies (Farvid et al. 2016). Further, the consumption of many naturally occurring components in high amounts reduces the relapse and increases the rate of survival considerably in breast cancer patients (Nechuta et al. 2012). Natural dietary compounds and their bioactive ingredients effectively inhibit breast cancer by downregulating ER-α release and activity; decreasing growth, angiogenesis, and metastasis of tumor cells of the breast; initiating cell cycle arrest and apoptosis; and finally sensitizing the breast cancer cells to radiotherapy and chemotherapy (Varinska et al. 2015). Few of the naturally occurring dietary components and their downregulatory effects on breast cancers are shown in Fig. 7.3.

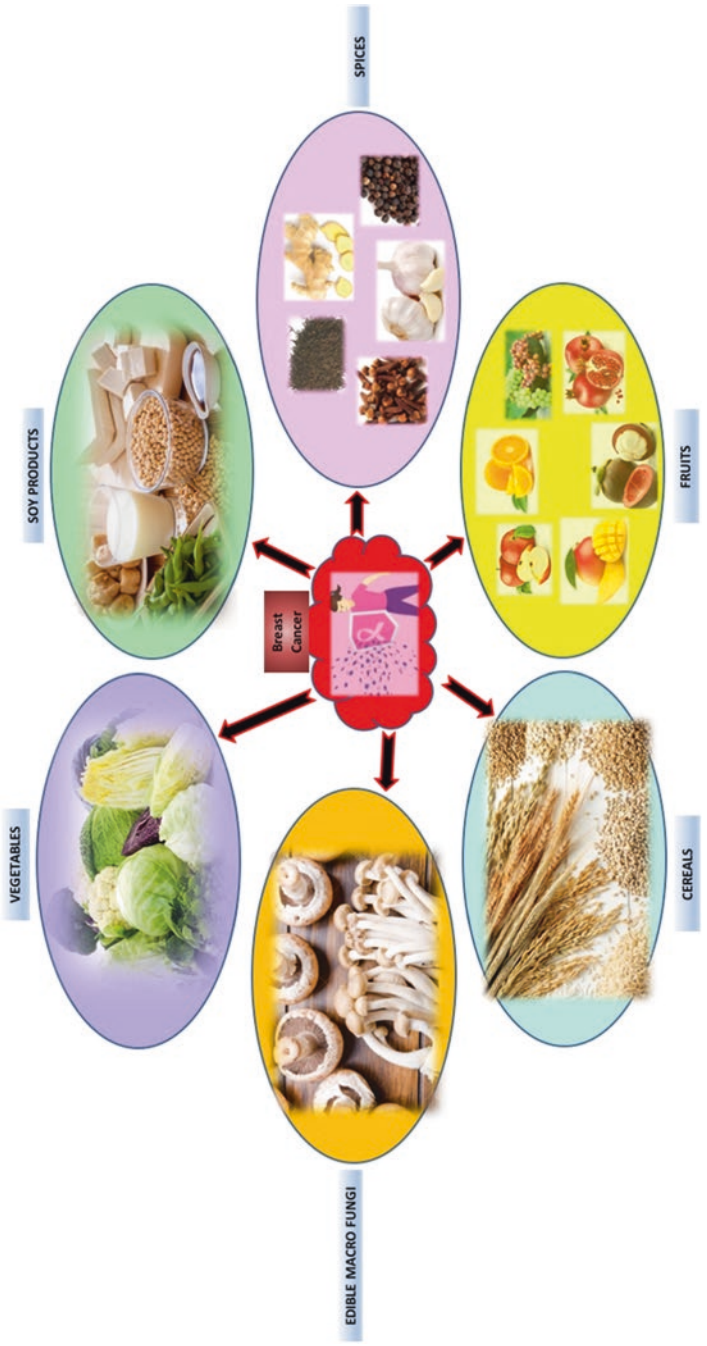

Fig. 7.3 Dietary natural products and their inhibitory effects on breast cancer

7.6.1 Soy Products

Soy and its products possess several health benefits, including lowering of the incidence of coronary heart diseases (Zhang et al. 2003), diabetes (type 2) (Mueller et al. 2012), and tumors of the breast (Dong and Qin 2011). Soy products have been extensively consumed for centuries in the Asian subcontinent. A recent meta-analysis study emphasized that soy, being a rich source of isoflavones (genistein and daidzein), significantly reduces the risk of breast cancer via the ER-based signaling pathways as they bear structural likeliness to 17-β-estradiol. A principal soy isoflavone, genistein, binds to both ERα and ERβ, where the ERα/ERβ proportion is considered as a foreboding beacon for breast cancers, and it eventually regulates the progression and mitochondrial functions of cancerous cells in a ERα/ERβ ratio-dependent manner by stimulating mitochondrial functionality and cell cycle arrest in T47D cells (low ERα/ERβ ratio), but without influencing MDA-MB-231 (ER-negative) cells and MCF-7 (high ERα/ERβ ratio) (Pons et al. 2014). Genistein in combination with other anticancer agents like tamoxifen, paclitaxel, or cisplatin exhibits predominant synergetic effect (ERα/ERβ ratio dependent) in T47D cells when compared to MDA-MB-231 and MCF-7 cells. Estrogen influences the soy diet treatment of breast cancer. A study suggested that lower level of estrogen encourages breast cancer progression; however, high levels of estrogen significantly inhibit breast cancer. Notably, this influence might happen only during the early phase of breast cancer (Zhang et al. 2012). Various studies prove that soy isoflavones not only hinder the growth of ER-negative MDA-MB-231 breast cancers but also exhibit antibreast cancer effect via diverse ER-independent aspects (Li et al. 2008; Magee et al. 2014). It has been proven fact that soy products (isoflavones) initiate apoptotic cell death in both ER^+ and ER^- breast cancer cells. For example, a long-duration fermented water-soluble extract of Korean soybean, doenjang arrested the cell cycle and hindered proliferation leading to apoptotic cell death in breast cancer cells (Seol et al. 2016). Genistein inhibited growth and triggered apoptosis in T47D-C3 and MCF-7 cells by downregulating the protein—phosphatase 2A (overexpressed oncogene inhibitor in breast cancer) (Zhao et al. 2016). Genistein inactivated IGF-1R/p-Akt signaling pathway, thereby suppressing the expression of Bcl-2/Bax mRNA leading to apoptosis in MCF-7 cells (Chen et al. 2015). Apoptosis in MCF10CA1a cells was induced by another similar compound, 6,7,4′-trihydroxyisoflavone, a daidzein metabolite which upregulated DR4 and downregulated XIAP expressions eventually causing PARP cleavage. It also regulates cyclins and cyclin-dependent kinases (CDKs) and triggers cell cycle arrest in the S and G2/M phases (Lee and Lee 2013). A fungus bio-transformed extract of a soybean effectively induced cell death in MCF-7 cells, which in turn led to caspase-3 and proapoptotic molecule upregulations (Stocco et al. 2015). Epigenetic alterations like inhibition in the methylation of DNA and enhanced expression of certain tumor suppressor genes in the breast cancer cells are also caused by soy isoflavones, which are proven signs of anticancer effects (Xie et al. 2014). A more complex manner of DNA damage response and cell cycle modulation stalling the proliferation of TNBC cells by genistein was quantified through phosphoproteomic studies. Soy isoflavones block

various pro-survival signaling in breast cancers and also block the effective functioning of NF-κB through Nocth-1 signaling pathway, thereby inhibiting the exponential growth of MDA-MB-231 cells dose dependently. Genistein downregulates the Hedgehog-GLI 1 signaling pathway, which in turn decreases the stem-like cell population (both in vitro and in vivo) in breast cancers (Fan et al. 2013).

7.6.2 Fruits

Several studies confirm the fact that fruits are an eternal source of polyphenols possessing antioxidant activity to minimize cancer risks (Lee and Lee 2013; Li et al. 2016; Fu et al. 2010; Fu et al. 2011; Akhtar and Swamy 2018a, b). Chiefly, pomegranate (*Punica granatum L.*) having a nick name of "nature's power fruit" has been consumed for centuries as it harbors several medicinal qualities. Ellagitannins are the major polyphenols occurring in pomegranate, and it has been proven that it contains high antioxidant and excellent anti-inflammatory properties (Legua et al. 2016). Apoptosis and cell cycle arrest in G2/M phase were found to be promoted by pomegranate extract in the MCF-7 cells (breast cancer), thereby affecting homologous recombination and sensitizing the cancer cells to double strand breaks (Shirode et al. 2014). Even the hydrophilic fraction extracted from the seed oil of pomegranates brought down the viability of 4,4′-methylenedianiline (MDA)-MB-231 and MCF-7 breast cancer cells significantly by triggering cell cycle arrest in the G0/G1 phase (Costantini et al. 2014). Proapoptotic and antiproliferative effects were seen in 7,12-Dimethylbenzanthracene (DMBA)-inflicted rat mammary tumorigenesis when treated with pomegranate extract. The possible mechanism of action was deduced to be the retarded ER and Wnt/−catenin signaling pathways (Mandal and Bishayee 2015). Studies show that even the fermented juice and extracts of seed oil hinder the infiltration and progression of breast cancer cells in humans by stalling the expressions of Rho A (Ras homolog gene family, member A) and Rho C proteins (Khan et al. 2009; Adams et al. 2010). The aqueous form of pomegranate extract consists of ellagitannins and phenolic acids (bioactive components), and the lipid extract from the seeds contains conjugated octadecatrienoic acids. Hence, compounds derived from ellagitannin prove to be a promising goal in the inhibition of estrogen-responsive breast tumors by inhibiting the aromatase activity and invasion of cancerous cells (Ismail et al. 2016; Izabela et al. 2018).

A tropical fruit acknowledged as "queen of fruits" is the mangosteen (*Garcinia mangostana L.*), and its crude methanolic extract has exhibited to inhibit the growth of the cell line, SKBR3 (human breast cancer), by initiating apoptosis. Likewise, the phenolic compounds from the fruit's pericarp displayed cytotoxic behavior on MCF-7 breast cancer cells (Gutierrez-Orozco and Failla 2013). Anticancer, antioxidant, and anti-inflammatory activities are a few among many bioactivities exhibited by α- and γ-mangostin, one of the most important xanthones among the 12, extracted from mangosteen's pericarp. Among the xanthones, α-mangostin, γ-mangostin, garcinone-D, and garcinone-E exhibited anti-aromatase effects in a dose-dependent manner in the SK-BR-3 breast cancer cells (Balunas et al. 2008). α-mangostin has

the ability to stimulate apoptotic cell death in the breast cancer cell line T47D by altering the HER2/PI3K/Akt and MAPK signaling pathways (Kritsanawong et al. 2016; Kurose et al. 2012) and induces apoptosis (mitochondria-mediated) and variations in cell cycle in MDA-MB-231 cells having mutated p53 gene. The treatment of α-mangostin at a dose of 20 mg/kg/day ratio suppressed tumor volume, increased survival rate, and increased lymph node metastases in mice with mammary tumors (Shibata et al. 2011). Similarly, panaxanthone (80% α-mangostin and 20% γ-mangostin) extracted from mangosteen's pericarp displayed mammary tumor suppression (volume) and low progression of lung and lymph node metastasis through elevated apoptosis-mediated cell death, antiproliferation, and antiangiogenesis (Aizat et al. 2019).

Apple is a very commonly consumed integral fruit of the human diet. Flavonoids are abundantly present in its peel and pulp. Hence, it can effectively control the growth of cancer cells. In a study, it was shown that apple peel and pulp extract significantly inhibited the MCF-7 (breast cancer) cells with an IC_{50} value of 58.42 ± 1.39 mg/ml and 296.06 ± 3.71 mg/ml, respectively (Yang et al. 2015). Pelingo is an apple cultivar, and its juice contains enormous polyphenol contents. It was shown to exhibit antiproliferative behavior in both MDA-MB-231 and MCF-7 cells, and further it can notably downregulate in vitro tumorigenesis and propagation of human breast cancer cells and could supply natural bioactive nonnutrient compounds with remarkable chemopreventive activity (Schiavano et al. 2015). Besides, MDA-MB-231 and MCF-7 cells showed antiproliferative effect upon treatment with 10–80 mg/ml ($p < 0.05$) concentration of apple extract. To be more precise, apple extract was capable of initiating cell cycle arrest at G1 phase by lowering the levels of cyclin D1 and Cdk4 proteins in the MCF-7 cells (Angelo et al. 2017).

Grapes and its one of the most common and important products, wine, are considered to be healthy and are consumed worldwide. Studies have shown that a BALB/c mouse implanted with 4 T1 cells (breast cancer cells) had significantly reduced lung metastasis rate when treated with grape skin extract in the concentration scale of 0.5–1.0 mg/ml in drinking water (Yang and Liu 2009). The proposed mode of action was elucidated to be due to the action of polyphenols of the grape skin, which stopped the migration of 4 T1 cells, and subsequently halted the PI3k/Akt and MAPK pathways. Similarly, the extract from grape seeds inhibited the progression of the extremely metastatic MDA-MB231 cells by hindering β-catenin release and localization, lowering the exhibition of fascin and NF-κB, and altering MMP-9, MMP-2, and urokinase-type plasminogen activator (uPA) activities (Dinicola et al. 2014). A selective cytotoxicity like damage of the membrane, dysfunctional mitochondria, and G2/M cell cycle arrest was observed in MCF-7 cells when treated with polyphenol fraction of red grape wine (Hakimuddin et al. 2006). Mango (*Mangifera indica* L.), "The king of fruits," is a tropical fruit abundant in polyphenolic constitutes like gallic acid and gallotannins (Zhao et al. 2014). Studies have confirmed the cytotoxic effects of mango polyphenols. A study reported the suppression of tumor volume by 73% in BT474 cells under in vitro conditions. Also, in mice model of BT474 xenograft, the progression of cancer was inhibited in comparison to control animals when treated with mango polyphenols. This tumor

inhibition was believed to be owing to the influence of polyphenols on PI3K/AKT pathway and miR-126 (Banerjee et al. 2015). Further, evidence states that the extracts from the peel of Nam Doc Mai mango have the paramount quantity of polyphenols, and hence it significantly inhibited the sustenance of MCF-7 cells with IC_{50} value of 56 μg/ml. Also, cell death was observed in MDA-MB-231 cells (Hoang et al. 2015). Similarly, apoptosis was noticed in both MDA-MB-231 and MCF-7 cells along with increased levels of proapoptotic proteins and diminished levels of antiapoptotic proteins during the treatment with ethanolic extract of mango seed kernels (Abdullah et al. 2015; Huang et al. 2018).

7.6.3 Cruciferous Vegetables

Cruciferous vegetables comprise broccoli, cauliflower, watercress, and brussel sprouts that are cultivated and consumed worldwide. They exhibit antibreast cancerous behavior on experimental models as they possess higher levels of glucosinolates. Myrosinase (an enzyme) is released when the vegetable is cut or chewed and the glucosinolates present in the vegetable degrade to form isothiocyanates, which comprises compounds like benzyl isothiocyanate, phenethyl isothiocyanate, and sulforaphane that are known to exhibit chemopreventive activities against breast cancer (Kang et al. 2009; Liu and Lv 2013). Apoptosis and mitochondrial fusion inhibition were recorded in breast cancer cells when benzyl isothiocyanate (BITC) was administered (Kim et al., 2010; Kim and Singh 2010). Bax and Bak (proapoptotic proteins) and Bcl-2 and Bcl-xL (antiapoptotic proteins) were upregulated and downregulated, respectively, along with caspase-3, caspase-8, and caspase-9 cleavage and reactive oxygen species (ROS) production. The normal cancerous cells of the breast and xenografts of MDA-MB-231 exhibited death by autophagy (FoxO1-mediated) when treated with BITC (Xiao et al. 2012). In both MCF-7 and MDA-MB-231 cells, the stimulation of signal transducer and transcription 3 was suppressed when exposed to BITC, indicating the antioncogenic property. Similarly, other in vitro study focused on vernodalin (sesquiterpene lactone), an active pure component isolated from bitter cumin seeds possessing cytotoxic effects and induces apoptosis by activating FOXO3a-mediated apoptosis in both MDA-MB-231 and MCF-7 cell lines. Further, this study was validated with in vivo experiments with LA7 mammary tumor model, which reveals that vernodalin has the potential to downregulate the PI3K/Akt/FOXO signaling pathway thereby inhibiting mammary breast tumor (Ananda Sadagopan et al. 2015). Similar to vernodalin, phenethyl isothiocyanate (PEITC) is another naturally occurring isothiocyanate, which can potentially inhibit cancer (breast) cell growth. Reports reveal apoptosis independent of p53-upregulated modulator of apoptosis (PUMA) in BRI-JM04 cells (breast cancer) facilitated by the B-cell lymphoma 2 interacting mediator of cell death (Bim). It is also proven that PEITC can effectively suppress adhesion, aggregation, progression, and metastasis in both MDA-MB-231 and MCF-7 cells by HIF-1α modifications and also prolong the survival rates in rats (tumor-free) along with reduction in tumor frequency by N-methyl nitrosourea (NMU) (Hahm and Singh 2012; Sarkar et al. 2016).

Vegetables from the Brassica family are rich sources of indole-3-carbinol (I3C), which are proven natural anticarcinogenic agents, especially against breast cancer as they bind to the ER and inhibit its activity or stall cycle progression, metastasis, and apoptosis (estrogen-independent). In the cell lines MDA-MB-468 and MCF-7, the administration of I3C at 50–100 μM concentrations effectively lowered cell adhesion, propagation (in vitro), and lung metastasis (in vivo) along with BRCA1 and E-cadherin/catenin complex's upregulation (Meng et al. 2000). Studies have also proven that I3C can effectively stop cancer cell migration by hindering the process of epithelial–mesenchymal transition (EMT). Also, it downregulated the focal adhesion kinase (FAK) expression (Ho et al. 2013) and inhibits the infiltration of breast cancer cells by arresting the ERK/Sp1-assisted gene transcription through I3C-directed inhibition of MMP-2 (Hung and Chang 2009). Bone metastasis due to breast cancer is inhibited upon I3C administration, which affects the MMP-9 and CXC chemokine receptor (CXCR4) expressions by downregulating the nuclear factor (NF-κB) signaling pathway. Antiproliferative effects of I3C were seen in MCF-7 cells (estrogen-sensitive), where ER was downregulated to result in the downregulation of insulin receptor substrate 1 (IRS1) and insulin-like growth factor 1 receptor (IGF1R) (Marconett et al. 2012). Reports also confirm that stress fibers and focal adhesions were formed when Rho kinase activity was enhanced upon I3C treatment which eventually led to the suppressed motility in MDA-MB-231 cells (Wei et al. 2016).

7.6.4 Spices

Since time immemorial, various spices have been extensively used in the preparation of traditional medicines and in food seasoning. Each spice has its own bioactive constituents, and some of the examples include gingerols and shogaols present in ginger, thymoquinone present in black cumin, and organosulfur components found in garlic. All these compounds possess anticancer activity against breast cancer.

For thousands of years, *Zingiber officinale* acknowledged as ginger, one among the well-used spices having excellent medicinal properties. Recent studies have proven that ginger possess an effective antibreast cancer agent, gingerol. The methanolic extract of ginger when treated to MDA-MB-231 cells controlled the proliferation and colony formation efficiently in a both dose- and time-dependent manner (Ansari et al. 2016). With Bax's stimulation and suppression of cyclin D1, Bcl-X, CDK-4, Bcl-2 proteins, survivin, Mcl-1, and NF-κB, apoptosis was effectively observed in both MDA-MB-231 and MCF-7 cells with the treatment of ginger extract. It also effectively hindered c-Myc and hTERT expressions, which are the most important molecular targets of tumors (Elkady et al. 2012). In addition, 10-gingerol, an important component of ginger, has been proven to hinder the proliferation and spread of MDA-MB-231 breast cancer cells by cell cycle arrest in the G1 phase via downregulation of cyclin-dependent kinases and cyclins and suppressing Akt and p38 (MAPK) activity leading to the inhibition of cancer cell invasions (Martin et al. 2017). Similarly, another study also showed that 6-gingerol administered to MDA-MB-231 cells hindered cell migration by suppressing the release and

behavior of MMP-2 and MMP-9 (Joo et al. 2016). Further, studies have revealed that the incidences of vomiting and severity of nausea reduced when ginger was orally supplemented to breast cancer patients (Marx et al. 2014). It is also reported that even the inhalation of ginger essential oil can reduce nausea during the acute phase of cancer. Overall, aromatherapy using ginger greatly influenced the basis for improvements in global health status and appetite loss (Arslan and Ozdemir 2015; Evans et al. 2018).

Another equally important spice is garlic (*Allium sativum*) used extensively in folk medicines to treat multiple ailments. Research reports from hospital-based case–control study have shown that garlic consumption along with other *Allium* vegetables can minimize the occurrence of breast cancer due to the presence of organosulfur components like diallyl trisulfide, allicin, diallyl disulfide, and S-allyl mercaptocysteine, and these observations reported that the intake of garlic was predominantly accompanied with a lower BC risk (Yun et al. 2014; Pourzand et al. 2016). A chief organosulfur component of garlic oil is diallyl disulfide (DADS), effectively initiates apoptotic cell death in MCF-7 cells by inhibiting the process of deacetylation of histone, stress-activated protein kinases/Jun amino-terminal kinases (SAPK/JNK), extracellular regulatory kinase (ERK) suppression, and p38 pathway activation (Lee et al. 2015a, b). Positive results were seen even in TNBC cases, when DADS inactivated the β-catenin signaling pathway leading to inhibited growth and metastasis (Huang et al. 2015). Another similar compound, diallyl trisulfide (DATS), is also an efficient inducer of apoptosis as it generates ROS excessively leading to the upregulation of AP-1 and JNK eventually resulting in apoptotic cell death in MCF-7 cells and tumor xenografts. The elevated amounts of cyclin B1, FAS, Bax, p53, and cyclin D1 expression and suppression of release levels of Bcl-2 and Akt are some of the possible mechanisms of action of DATS to initiate apoptotic cell death in MCF-7 cells. Even in the aggressive TNBC cells, DATS suppressed ERK/MAPK and NF-κB signaling pathways by inhibiting MMP2/9, ultimately leading to disrupted migration and invasion of the cancerous cells (Kiesel and Stan 2017; Puccinelli and Stan 2017).

For nearly 1400 years, *Nigella sativa* popularly known as "black cumin" has been exploited for its medicinal properties against the treatment of various ailments. However, its anticancer effects are being extensively studied in recent times. An in vitro study involving MCF-7 cells showed that the supercritical CO_2 extract from black cumin has antiproliferative, antimetastatic and proapoptotic effects by altering the p53 and caspase pathways (Baharetha et al. 2013; Alhazmi et al. 2014). The major bioactive compound isolated from black cumin seeds was thymoquinone (TQ), which is believed to be an accomplished chemopreventive and chemotherapeutic agent known to suppress tumor proliferation and to enhance apoptotic cell death in breast cancer containing xenograft mice via ROS generation and p38 phosphorylation. Other suggested antiproliferation mechanistic ways of action of TQ in breast cancer cells is through production of prostaglandin E2 via PI3K/p38 kinase pathway, COX-2 expression, and PPAR-γ activation pathway modulation (Woo et al. 2013; Asaduzzaman Khan et al. 2017). Thus, spices like black cumin, ginger, and garlic among others possess anticancer properties against breast cancer, and

their bioactive components like diallyl trisulfide and diallyl disulfide found in garlic and shogaols and gingerols present in ginger are being extensively studied for their anticancer therapeutic properties.

7.7 Synergistic Effects of Dietary Natural Products with Anticancer Therapies

The most regularly used treatment regimens for cancer nowadays include chemotherapy and radiotherapy, which always leaves behind adverse toxic effects and drug resistance leading to the failure of chemotherapy and relapse. However, synergistic effects were observed when chemotherapy or radiotherapy was accompanied by natural dietary compounds and their bioactive constituents as they enhance their remedying effects and reduce the possible after-effects. To quote as an example, genistein and doxorubicin when given in combination exhibited synergistic effect in MCF-7/Adr cells, increasing the intracellular build-up of doxorubicin and lowering the expressions of HER2/neu (Xue et al. 2014). Similarly, genistein–centchroman (a selective estrogen receptor modulator) combination selectively acted on the breast cancer cells on humans by increasing the cytotoxicity in comparison to each drug when used separately and most importantly did not affect the surrounding human mammary nontumorigenic epithelial cells (Kaushik et al. 2016). Studies show that pomegranate extract induced apoptosis in both susceptible and TAM-resistant MCF-7 cells when given along-side tamoxifen causing tamoxifen-induced suppression on cell viability (Rahmani et al. 2017). 3,3′-Diindolylmethane (DIM) and paclitaxel combinedly inhibited Her2/Neu human breast cancer cell growth by regulating the Her2/Neu receptor and ERK1/2 (its subsequent target) and also enhanced apoptosis via Bcl-2/PARP mitochondrial pathway (Wang et al. 2016), again displaying synergistic effects. ER-negative MDA-MB-231 and ER-positive MCF-7 cells displayed reduced viability when apoptosis was initiated upon the synergistic action (treatment) of TQ and tamoxifen (Ganji-Harsini et al. 2016). Similar results of inhibited breast cancer cell growth via apoptosis were observed in both culture studies and in mice when TQ–paclitaxel combination was administered (Sakalar et al. 2016).

A key arabinoxylan MGN-3 extracted from rice bran is known to harbor umpteen immunomodulatory properties against the diverse immune cells like macrophages, NK cells, T and B cells, and dendritic cells (DC). It is also a potent antitumor agent which effectively sensitizes both metastatic 4 T1 (murine BCC) and nonmetastatic MCF-7 (human BCC) cells to paclitaxel eventually resulting in the enhanced susceptibility of both the cell lines by over 100-fold to paclitaxel. Systematically, MGN-3 synergistically functions with paclitaxel by inducing apoptosis, hindering cell multiplication, and facilitating DNA damage in 4 T1 cells. These studies confirm that MGN-3 can be supplemented with food along with chemotherapeutic drugs like paclitaxel in treating metastatic breast cancers (Ghoneum et al. 2014). The possible mode of action of the various dietary natural products as antibreast cancer agents under both in vitro and in vivo conditions is listed in Table 7.5.

Table 7.5 In vitro and in vivo effects of dietary natural products against breast cancer

Natural product	Constituents	Study type	Possible mechanisms of action	References
Soy and its product				
Soy	Genistein	In vitro	Inducing cell cycle arrest Improving mitochondrial functionality Regulating oxidative stress, uncoupling proteins, antioxidant enzymes, and sirtuin Enhancing effects of anticancer drugs	Nadal-Serrano et al. (2013), Pons et al. (2014), Pons et al. (2016)
Soy	Genistein	In vitro	Inducing apoptosis through downregulation of the cancerous inhibitor of protein phosphatase 2A The inactivation of the IGF-1R/p-Akt signaling pathway	Chen et al. (2015), Zhao et al. (2016)
Soy	Genistein	In vitro	Inhibiting cancer cell growth through modulating the DNA damage response and cell cycle Inhibiting cancer cell growth through inhibiting activity of NF-κB via the Nocth-1 signaling pathway	Pan et al. (2012), Fang et al. (2016)
Soy	Genistein	In vitro and in vivo	Decreasing breast cancer stem-like cell population through hedgehog pathway	Fan et al. (2013)
Soy	Daidzein and equol	In vitro	Inhibiting the invasion through the downregulation of MMP-2 expression	Magee et al. (2014)
Fruits				
Pomegranate	Luteolin, ellagic acid, punicic acid	In vitro	Inhibiting growth, increasing adhesion, and decreasing migration of breast cancer cells	Rocha et al. (2012)
Pomegranate	Ellagitannins, phenolic acids, conjugated octadecatrienoic acids	In vitro	Inhibiting invasion and motility of cancer cells by inhibiting RhoC and RhoA protein expression	Khan et al. (2009)

(continued)

Table 7.5 (continued)

Natural product	Constituents	Study type	Possible mechanisms of action	References
Pomegranate	Ellagitannin-derived compounds	In vitro	Inhibiting aromatase activity and cell proliferation	Adams et al. (2010)
Mangosteen	Garcinone D, garcinone E, α-mangostin γ-Mangostin	In vitro	Dose-dependent antiaromatase activity	Balunas et al. (2008)
Mangosteen	α-Mangostin	In vivo and in vitro	Increasing survival rates and suppressing tumor volume and the multiplicity of lymph node metastases Inducing apoptosis and cell cycle arrest	Shibata et al. (2011)
Mangosteen	Panaxanthone	In vivo and in vitro	Suppressing tumor volumes and decreasing the multiplicity of lung metastasis and lymph node metastasis Inducing apoptosis	Doi et al. (2009)
Citrus fruit	Polysaccharides	In vitro	Inhibiting angiogenesis and cell migration	Park et al. (2016)
Citrus fruit	Naringin	In vitro and in vivo	Inhibiting growth potential by targeting β-Catenin pathway Inhibiting cell proliferation and promoting cell apoptosis and G1 cycle arrest through modulating β-catenin pathway	Li et al. (2013a, b)
Apple	Polyphenol	In vitro	Inhibiting tumorigenesis of preneoplastic cells by suppressing colony formation and ERK1/2 phosphorylation	Schiavano et al. (2015)
Apple	Pectic acid	In vitro and in vivo	Inducing apoptosis and inhibiting cell growth preventing tumor metastasis mice via overexpression of P53	Delphi et al. (2016)
Grape	Polyphenols	In vivo and in vitro	Inhibiting migration by blocking the PI3k/Akt and MAPK pathways	Sun et al. (2012)

(continued)

Table 7.5 (continued)

Natural product	Constituents	Study type	Possible mechanisms of action	References
Grape	Anthocyanin	In vitro	Decreasing invasion, migration, and bone turnover, via inhibiting expression of snail and phosphorylated STAT3 and abrogating Snail-mediated CatL activity	Burton et al. (2015)
Mango	Polyphenolics	In vitro and in vivo	Showing cytotoxic effects reducing the tumor volume by regulating the PI3K/AKT pathway and miR-126	Banerjee et al. (2015)
Mango	Pyrogallol	In vitro	Inhibiting proliferation through mediating the AKT/mTOR signaling pathway	Nemec et al. (2016)
Cruciferous Vegetables	Benzyl isothiocyanate	In vitro and in vivo	Suppression of uPA activity and of Akt Signaling Suppression on EMT process Inducing FoxO1-mediated autophagic death	Kim et al. (2012), Xiao et al. (2012)
Cruciferous Vegetables	Indole-3-carbinol	In vitro and in vivo	Suppressing metastasis through upregulation of BRCA1 and E-cadherin/catenin complexes Suppressing EMT process and Downregulating FAK expression Inhibition on MMP-2 expression Inhibiting CXCR4 and MMP-9 expression by downregulation of the NF-κB signaling pathway	Meng et al. (2000), Rahman et al. (2006), Hung and Chang (2009), Ho et al. (2013)
Spices				
Garlic	Diallyl disulfide	In vitro and in vivo	Suppression of the SRC/Ras/ERK pathway Inactivation of the β-Catenin signaling pathway	Xiao et al. (2014), Huang et al. (2015)

(continued)

Table 7.5 (continued)

Natural product	Constituents	Study type	Possible mechanisms of action	References
Garlic	Diallyl trisulfide	In vitro and in vivo	Inducing apoptosis through overproduction of ROS and subsequent Activation of JNK and AP-1 Upregulating FAS, Bax, and p53, and downregulating Akt and Bcl-2	Malki et al. (2009), Na et al. (2012)
Black cumin	Thymoquinone	In vitro and in vivo	Inducing apoptosis through inhibiting Akt phosphorylation Inducing p38 phosphorylation via ROS generation	Rajput et al. (2013), Woo et al. (2013)
Clove	Eugenolol	In vitro and in vivo	Inhibiting growth and proliferation, inducing apoptosis through targeting the 2F1/ surviving pathway	Al-Sharif et al. (2013)
Black pepper	Piperine	In vitro and in vivo	Inhibiting growth, motility and metastasis Inducing apoptosis	Do et al. (2013) Greenshields et al. (2015)

7.8 Conclusion and Future Prospects

The outcomes from varied clinical trials have not been eminently reported for any breakthrough development in cancer treatment regimens from the uptake of phytochemicals. However, many extensive and through preclinical studies claim significant anti-inflammatory, proapoptotic, antiproliferative, antimetastatic, antiangiogenic, and cytotoxic properties of phytochemicals against mammary tumors. In order to carry out such proficient clinical researches, specialized knowledge is required to create, explore, and implement various strategic approaches on aspects like individualized patient profiling, disease modeling scenario, multiomic diagnostics, and patient stratification by phenotyping/genotyping methods. Among the many, very few dietary natural products like cruciferous vegetables, soy, and citrus fruits reduce the risks of breast cancer considerably. Various epidemiological studies have reported the successful antiproliferative activity of dietary supplements and their bioactive compounds. To establish this, many research findings have reported that most dietary natural products are eminent sources for treating and terminating breast cancers. Ellagitannins from pomegranate, genistein and daidzein from soy, naringin from citrus fruits, gingerols and shogaols from ginger, mangostin from mangosteen, thymoquinone from black cumin, isothiocyanates from cruciferous vegetables, and organosulfur compounds from garlic, tropical fruits like mango and grapes, cereals, and many edible macrofungi are few among the many available natural dietary

compounds and their respective bioactive components, which have been reported to effectively treat and cure breast cancers. The proposed mechanism of action of these compounds is through downregulation of angiogenesis, migration, proliferation and metastasis of tumor cells, dysfunctional (arrest) cell cycle, induction of apoptotic cell death, and sensitization of tumor cells to radiotherapy and chemotherapy. However, further exploration and efficient clinical trials of such natural dietary compounds and their corresponding bioactive components are the need of the hour in order to fabricate optimal conditions and design individualized treatment algorithms in a more cost-effective manner and easily approachable chemopreventive alternative strategies through the involvement of dietary phytochemicals in cancer therapeutics.

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Chromenes: Phytomolecules with Immense Therapeutic Potential

8

Mohd Kamil Hussain, Mohammad Faheem Khan, Shahnaaz Khatoon, Abdullah G. Al-Sehemi, and Mohammad Saquib

Contents

Abstract

Chromenes (benzopyrans) are privileged scaffolds that are widely distributed in a plethora of biologically active natural products, drugs and therapeutic leads. *2H*-Chromenes and their benzofused derivatives are extensively distributed in nature and are considered essential for the development of new therapeutic agents for a variety of diseases. The chromene nucleus is a vital constituent of various naturally occurring and synthetic molecules with a broad range of bioactivities, such as

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anti-vascular, anti-microbial, antioxidant, anti-tumour, antifungal, antiviral, anti-cancer, anti-HIV, anti-tubercular, anti-coagulant, anti-inflammatory, oestrogenic, analgesic, anti-helminthic, herbicidal, anti-convulsant and anti-spasmolytic activity. Chromene constitutes the fundamental skeleton of different types of natural alkaloids, coumarins, flavonoids, polyphenols, anthocyanins and tocopherols. Recently, naturally occurring chromenes, such as wittifurans A, D, E and F, gramniphenol C, F and G, (+)-psiguadial B, parvinaphthol C, busseihydroquinone E, anthopogochromane A–C, tuberatolide B, (*R*)-sargachromenol, (*S*)-sargachromenol, obovatin, chalcones, flemingins A–C, G and H, soulamarin, lindbergin E–F and flemiphilippinones C have been isolated from various plants. This chapter not only critically reviews the recent literature reports on chromene as a privileged scaffold in medicinal chemistry, particularly, 2,2-dimethyl-2H-chromenes, natural benzochromenes and fused chromenes, but also highlights the need for further research on reported and new chromene-based phytomolecules to evaluate their possible therapeutic applications, and toxicological and particular genotoxic profiles against a wide range of diseases, especially cancer, drug-resistant microbial infections and lethal viral diseases.

Keywords
Chromenes · Privileged scaffolds · Natural products · Phytochemicals · Medicinal chemistry

8.1 Introduction

Chromene (a bicyclic aromatic heterocyclic compound) consists of a benzene ring fused to oxygen-containing pyran ring (Majumdar et al. 2015). Although *2H*-chromene is a small molecule, variously substituted derivatives of chromenes have fascinated scientists, because of the diverse pharmacological activities they exhibit. *2H*-Chromene is also widely used as a versatile scaffold in pharmaceutical chemistry and drug innovation science. It forms the basic skeleton of a number of natural and synthetic molecules possessing diverse biological activities. *2H*-Chromenes are constituents of a variety of naturally occurring polyphenols, alkaloids, coumarins, flavonoids, tocopherols, anthocyanins, rotenoids, stilbenoids, flavanoids and chromene glycosides. Till date, a large number of *2H*-chromene motif containing natural products have been discovered and their pharmacological properties, biogenetic pathway and synthesis have been thoroughly studied (Ram et al. 2014). The chromene scaffold is an important constituent of a variety of naturally occurring and synthetic molecules with different pharmaceutical and biological properties, such as anti-vascular, anti-tumour, anti-microbial, anti-spasmolytic, anti-inflammatory, anti-oxidant, anti-coagulant, anti-HIV, oestrogenic, anti-helminthic, anti-tubercular, anti-fungal, anti-viral, anti-convulsant, herbicidal and analgesic activities (Fig. 8.1). Recently, chromenes such as wittifurans A, D, E

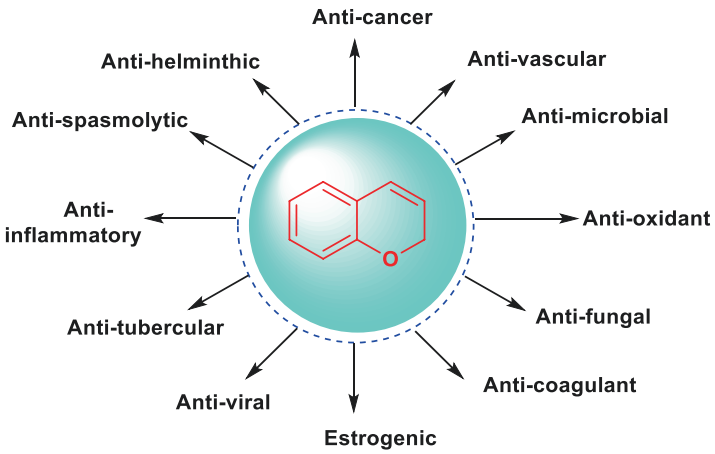


Fig. 8.1 Important pharmacological properties of chromenes

and F, gramniphenol C, F and G, (+)-psiguadial B, parvinaphthol C, busseihydroquinone E, anthopogochromanes A–C, tuberatolide B, *(R)*-sargachromenol, (*S*)-sargachromenol, obovatin, flemingins A–C, G, H, soulamarin, lindbergin E–F and flemiphilippinones C have been isolated from different parts of many a variety of herbs, shrubs and trees.

This chapter has been conceived to highlight the recent reports on natural chromenes as privileged scaffolds in medicinal chemistry and drug discovery, particularly 2,2-dimethyl-2H-chromenes, benzochromenes and fused chromenes.

8.2 Chromene and Its Therapeutic Potential

Chromene is a bicyclic aromatic heterocyclic compound, consisting of a benzene ring fused to an oxygen-containing pyran ring. It exists as different structural isomers resulting from the multiple relative positions the oxygen atom and the tetrahedral carbon atom can be present at, forming the four isomeric chromene molecular structures, namely, *2H*-chromene (**1**), *4H*-chromene (**2**) *1H*-isochromene (**3**) and *3H*-isochromene (**4**). Moreover, the fusion of benzene or naphthalene ring to different positions on the *2H*-chromene skeleton results in different classes of benzo- and naphthochromenes. The *2H*-chromene motif can fuse with the benzene ring at four possible positions (c, f, g, h), resulting in four types of benzochromenes, namely, benzo[*c*]chromene (**1a**), benzo[*f*]chromene (**1b**), benzo[*g*]chromene (**1c**) and benzo[*h*]chromenes (**1d**). Likewise, fusion of the *2H*-chromene with naphthalene results in four different types of naphthochromenes. In addition to benzochromenes, a variety of dibenzochromenes, such as dibenzo[*c,f*]cheomene (**1e**), dibenzo[*c,h*]chromene (**1f**), etc., have also been reported (Pratap and Ram 2014) (Fig. 8.2).

8.2.1 Naturally Occurring 2,2-Dimethyl-*2H*-Chromenes

Various substituted derivatives of *2H*-chromenes, such as methylripariochromene A (MRC) (**5**), acetovanillochromene (**6**) and orthochromene (**7**), were sequestered from chloroform ($CHCl_3$) extract of *Orthosiphon aristatus* leaves (Fig. 8.3). *O. aristatus* has been utilized in the traditional systems of medicine for treating diabetes and hypertension (Shibuya et al. 1999; Ohashi et al. 2000) (Fig. 8.3). MRC exhibit anti-hypertensive properties, such as a decline in cardiac output and vasodilatory action as well as diuretic action (Matsubara et al. 1999). Ageratochromene (**8**), a derivative of *2H*-chromene, was sequestered from *Ageratum mexicanum* Sims (Hlubucek et al. 1971) and *Ageratum conyzoides* L. (Dean 1963). Alloevodione (**9**)

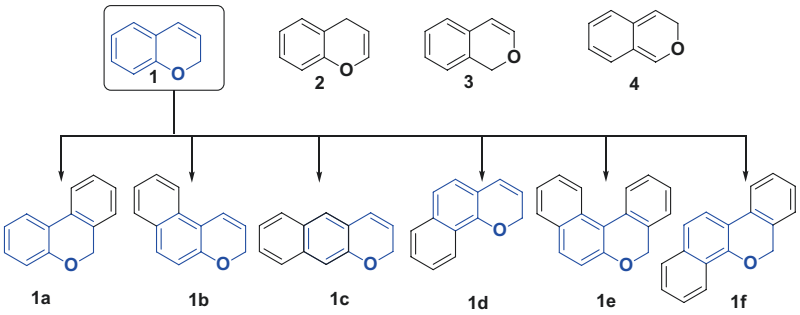


Fig. 8.2 *2H*-Chromene (1), *4H*-chromene (2), ***1H***-isochromene (3), *3H*-isochromene (4), benzo[*c*]chromene, (1a) benzo[*f*]chromene (1b), benzo[*g*]chromene (1c), benzo[*h*]chromene (1d), dibenzo[*c,f*]chromene (1e) and dibenzo[*c,h*]chromene (1f)

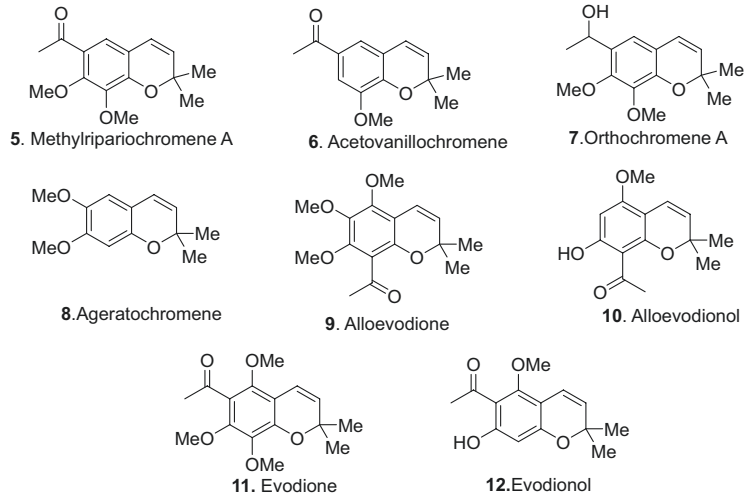


Fig. 8.3 Some bioactive 2,2-dimethyl-*2H*-chromene derivatives

is another natural chromene isolated from *Evodia elleryana* F. Muell. dry leaves (Kirby and Sutherland 1956). Alloevodionol (**10**) is also present in the leaves of *Medicosma cunninghamii* Hook F. (Rutaceae) (Kirby and Sutherland 1956). Evodione (**11**) is another derivative of 2,2-dimethyl-*2H*-chromenes, isolated from *Melicope lunu-ankenda*, which showed mild analgesic and moderate anti-inflammatory activities (Johnson et al. 2010). Evodionol (**12**) is related to evodione and is present in the leaf oil (1–3%) of *Medicosma cunninghamii* (Brophy et al. 2004) (Fig. 8.3).

8.2.2 Chalcone-Based 2,2-Dimethyl-2H-Chromenes

Lonchocarpin (**13**), a natural chalcone, was first isolated from the ether extracts of roots and seeds of *Lonchocarpus sericeus* (Dean 1963). Another chalcone, lonchocarpin reported from *Pongamia pinnata* roots, showed strong anti-cancer activity against six lung cancer cells (H226, H292, H460, H522, H1944 and SW1573). It also exhibited moderate anti-proliferative activity against four cancer cells, namely, H358, H1792, A549 and Calu-1. Lonchocarpin inhibits cell proliferation through the modulation of Bax/Caspase-9/Caspase-3 pathway. This molecule in vivo suppressed the growth of tumour in S180-bearing mice by 57.9%, 63.4% and 72.5% at a dose of 25, 50 and 100 mg/kg, respectively. These observations indicated that lonchocarpin can be a potential naturally occurring lead molecule for treating lung cancer (Chen et al. 2017) (Fig. 8.4).

Millepachine (**14**), a chalcone-based naturally occurring *2H*-chromene derivative, was first obtained from the *Millettia pachycarpa* plant, belonging to the Leguminosae family. It displayed strong anti-proliferative activity in different types of human cancerous cells, most notably in HepG2 cells (IC_{50} = 1.51 μM).

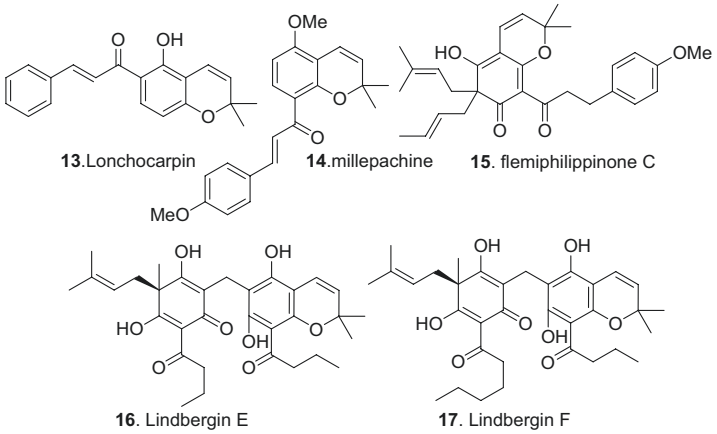


Fig. 8.4 Structures of some anti-cancer and anti-leishmanial 2,2-dimethyl-*2H*-chromene derivatives

Millepachine induced apoptosis via ROS–mitochondrial apoptotic pathway, and G2/M cell cycle arrest by inhibiting CDK1 activity, in human hepatocarcinoma cells (HepG2 cells) in vitro and in HepG2 tumour-bearing mice models in vivo (Wu et al. 2013) (Fig. 8.4).

Flemiphilippinones C (**15**), sequestered from *Flemingia philippinensis* roots, presented anti-cancer property against PC-3 cells (GI_{50} = 14.12 μM), Bel-7402 (GI_{50} = 1.91 μM) and CaEs-17 cells (GI_{50} = 2.58 μM). Flemiphilippinones C arrested the cell cycle (at the S/G2 phase) and stimulated apoptotic activity in hepatocellular carcinoma (Bel-7402) cells via mitochondria-mediated pathway (Kang et al. 2016). Socolsky and co-workers isolated lindbergin E (**16**), lindbergin F (**17**) and a prenylated acylphloroglucinol from the diethyl ether extract of *Elaphoglossum lindbergii*, a fern found in Argentina. Their leishmanicidal activity was investigated against the promastigotes of *L. Amazonensis* and *L. Braziliensis*, using amphotericin B as a control. Lindbergin E (**16**) and F (**17**) showed weaker leishmanicidal activity (IC_{50} of 13.6 and 29.9 μM, respectively) against *L. braziliensis*, as compared to the positive control, camphotericin B. In general, these molecules displayed better activity against *L. braziliensis*, as compared to *L. amazonensis*. Lindbergin E differs from lindbergin F only in the length of one of the acyl substituents. It was observed that the hexanoyl substituent greatly reduced the leishmanicidal activity, as compared to the butanoyl substituent (Fig. 8.4). 2,2-dimethyl-2H-chromene (**18**), chromane (**19**) and dibenzofuran fused *2H*-chromane (**20**) together with other reported composites were obtained from *Achyrocline satureioides* plant aerial parts (Casero et al. 2015). The anti-microbial effect of the isolated compounds was determined against a select set of Gram-positive and Gram-negative bacteria, in addition to a panel of yeasts and moulds using standard antibiotics, ampicillin and kanamycin as controls. Among these phytometabolites, compound **3** was found to be the most effective as an antibacterial agent. The dibenzofuran (**20**) showed inhibitory activity against both strains of *Staphylococcus aureus* and *Enteroccocus faecalis*, at concentrations equal to or higher than 32 and 64 μM, respectively (Fig. 8.5).

8.2.3 Natural Benzochromenes

Benzochromene ring systems are extensively distributed in naturally occurring compounds and are reported to possess a wide range of pharmacological

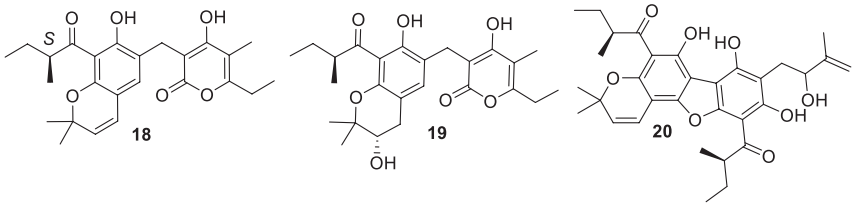


Fig. 8.5 Structures of some anti-microbial 2,2-dimethyl-*2H*-chromene derivatives

properties. A variety of natural benzochromene scaffolds have been isolated and characterized so far.

Two new benzo[*c*]chromenes, **21** and **22**, were sequestered from the plant roots of *Bourreria pulchra* (Family Boraginaceae) and evaluated for their anti-protozoan activity. Compound **21** showed a good activity against *Leishmania mexicana* (IC_{50} = 4.6 μg/ml) and *Trypanosoma cruzi* (IC_{50} = 7.5 μg/ml) (Erosa-Rjon et al. 2010), while compound **22** was found to be inactive. Δ^9-Tetrahydrocannabinols (**23)** and cannabinol (**24)** were isolated from *Cannabis sativa* (Marijuana plant) and were observed to display very good anti-microbial effect against different drug-resistant strains of *S. aureus* (XU212, ATCC25923, RN-4220, EMRSA-15, EMRSA-16 and SA-1199B) in a MIC range of 2–0.5 μg/ml (Appendino et al. 2008). Methyl-5,10-dihydroxy-7-methoxy-3-methyl-3-[4-methyl-3-pentenyl]-*3H*-benzo[*f*]chromene-9-carboxylate (**25)**, a homoprenylated benzochromene, was isolated for the first time from the solvent (hexane) extract of *Pentas bussei* plant roots. The decoction of this plant roots is used in the preparations of the traditional medications against dysentery, gonorrhoea and syphilis in Kenya (Bukuru et al. 2002) (Fig. 8.6).

Four compounds, including three new benzo[*f*]chromanes, namely, busseihydroquinones B–D (**26**, **27** and **28**), and a known homoprenylated dihydronaphthoquinone, were sequestered from dichloromethane (DCM) and methanol (1:1) extract of *P. bussei* roots. The isolated compounds and the crude root extract of this plant were tested for anti-plasmodial, antifungal and antibacterial actions against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* using known drugs, mefloquine and chloroquine, as positive control.

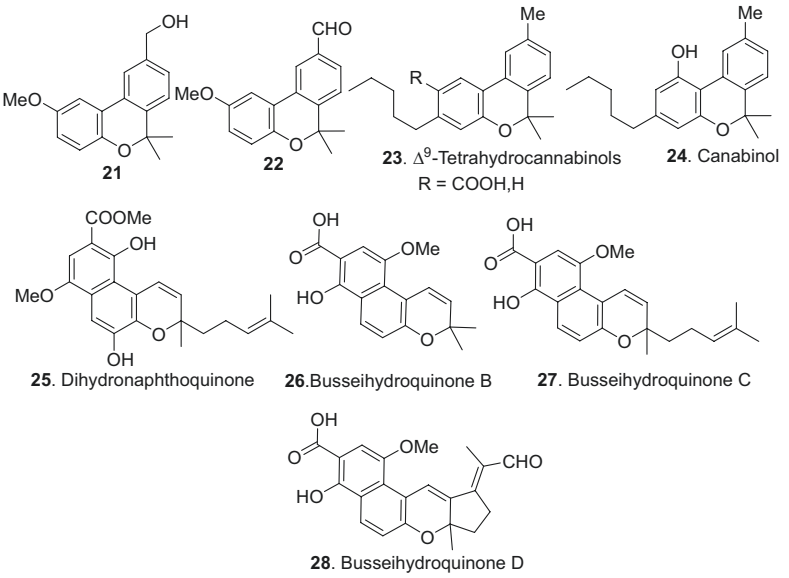


Fig. 8.6 Structures of some bioactive natural benzochromenes

However, only weak activity was observed against both the W2 and D6 strains. They also showed insignificant inhibition of fungal and bacterial strains (Endale et al. 2012) (Fig. 8.6).

Benzo[*g*]chromene ring systems are present in many naturally occurring compounds, which include many medicinally important natural products. These molecules have been found to be endowed with a variety of biological and medicinal properties. Quinone-based chlorinated benzo[*g*]chromene derivatives (**29–33**) have been extracted from CNQ-525 strain of actinomycete bacteria, isolated from the ocean sediments off La Jolla in the US state of California. These molecules were found to exhibit good efficacy against vancomycin-resistant *Enterococcus faecium* (VREF) and methicillin-resistant *Staphylococcus aureus* (MRSA) bacterial strains. They also displayed in vitro anti-proliferative action against different cancerous cells (Soria-Mercado et al. 2005) (Fig. 8.7). Four naphthoquinone-based benzo[*g*] chromene derivatives (**34–37**) were isolated through bioassay-mediated fractionation of *Catalpa ovate* stem-bark extract. These compounds also significantly inhibited tetradecanoylphorbol acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells (Fujiwara et al. 1998) (Fig. 8.7).

Benzo[*h*]chromenes display various bioactivities, including anti-cancer (Ough et al. 2005), anti-inflammatory (Moon et al. 2007) and anti-malarial (Elisa et al. 2005). The benzo[*h*]chromene moiety is present in various medicinally important natural products including β-lapachone (**38**), a naturally occurring benzo[*h*] chromene, isolated from the stem bark of the pink tabebuia tree *Tabebuia avellanedae*. Compound **38** exhibited anti-tumour, anti-viral and anti-trypanosomal activities in vivo (Docampo et al. 1977; Cruz et al. 1978; Schmidt et al. 1984). In particular, *β*-lapachone was reported to show very good anti-proliferative activity against different cancer cell lines such as prostate, lung, breast and promyelocytic leukaemia cell lines (Planchon et al. 1995). *β*-Lapachone also significantly suppressed the expression of NO and iNOS in alveolar macrophages and the growth of

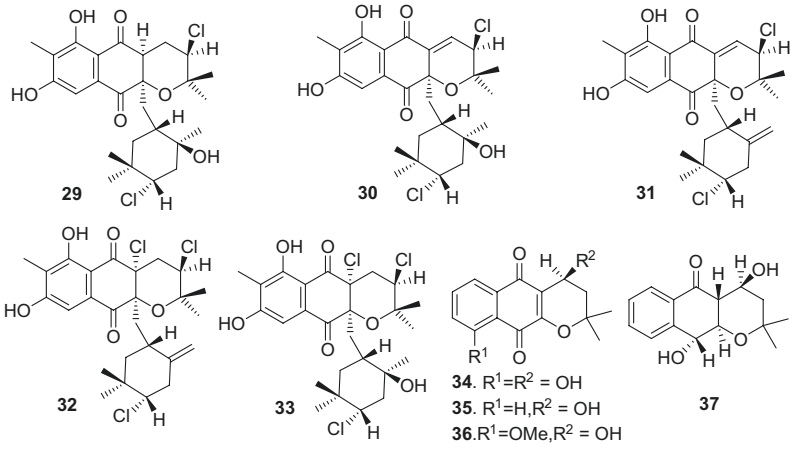


Fig. 8.7 Representative naphthoquinone-based bioactive natural benzo[*g*]chromenes

pre-established human pancreatic tumour xenografts in nude mice (Ough et al. 2005). In addition, *β*-lapachone also displayed potential anti-inflammatory activity by inhibiting the NF-kappa β activation, by retarding the IkappaBalpha degradation and downregulating the ERKs (extracellular signal-regulated kinases), MAPK (p38 mitogen-activated protein kinase) and PKB (protein kinase B) pathway (Moon et al. 2007).

Benzo[*h*]chromene derivatives, lapachenole (**39**) and dihydrolapachenole (**40**), have been isolated from the araguaney tree (*Tabebuia chrysantha*) (Burnett and Thomson 1968) (Fig. 8.6). In 2001, lapachenole isolated from *Avicennia rumphiana* was found to possess tumour-inhibiting activity, which was evaluated using an in vitro assay of TPA-induced EBV-EA activation in Raji cells (Itoigawa et al. 2001). Mollugin (**41**) and (**42**) were isolated from the *Rubia cardifolia*—a Chinese medicinal plant (Itokawa et al. 1983) and exhibited significant antitumour, mutagenic, recombinagenic (Kawasaki et al. 1992; Marec et al. 2001), as well as activity against hepatitis B virus (Ho et al. 1996). Mollugin showed strong suppression of the hepatitis B surface antigen secretion (IC_{50} = 2.0 μg/mL) in human hepatoma Hep3B cells (Ho et al. 1996) (Fig. 8.8). In addition, mollugin (**33**) was also found to strongly suppress the collagen-induced and arachidonic acid-induced platelet aggregation (Chung et al. 1994). Compounds **43a** and **43b** are other important benzo[h] chromenes which are *cis* and *trans* isomers and were isolated from the medicinal plant, *Pimelea longiflora*, which is endemic to tropical East Africa (Fig. 8.8).

8.2.4 Natural Chromenes Fused with Furan and Coumarin Moiety

Fused chromenes are extensively spread in the environment, especially found in a variety of medicinal plants. A number of fused chromenes, isolated from plant resources, were found to be beneficial in treating several diseases. Five furo[*f*] chromenes, namely, wittifurans A–C, F and G (**44–48**) (Fig. 8.9), have been extracted and identified from the stem-bark extract, i.e., the ethyl acetate fraction of the *Morus wittiorum* tree, and their structures were determined through

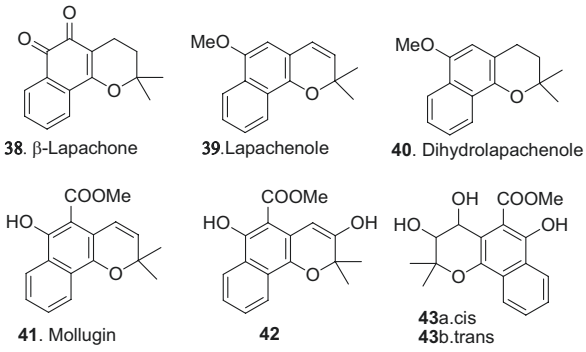


Fig. 8.8 Representative naturally occurring bioactive benzo[*h*] chromenes and chromane derivatives

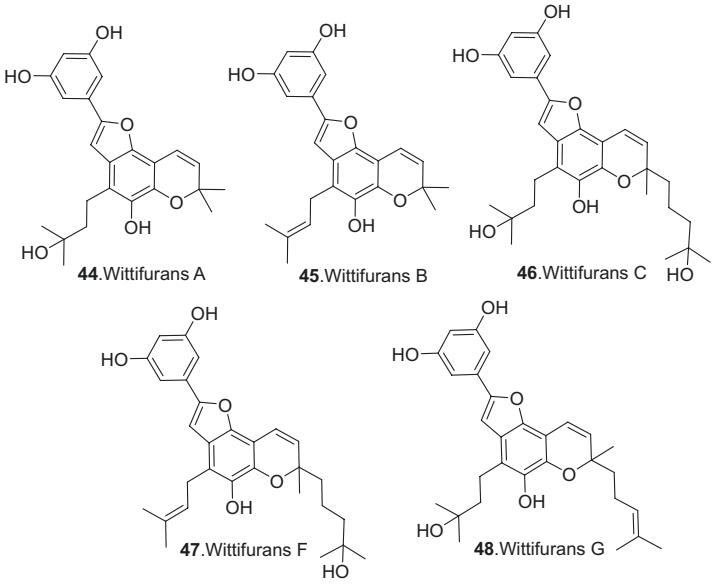


Fig. 8.9 Structures of furo[*f*]chromenes, also known as wittifurans A – C, F and G

spectroscopic studies. These compounds exhibited significant anti-inflammatory and anti-oxidant activities. Wittifurans A, C and G exhibited potent antioxidant activity in a Fe^{2+}-Cys system induced lipid peroxidation in liver microsomes, and also inhibited the production of malondialdehyde as well as the release of β-glucuronidase enzyme from rat's white blood cells, induced by PAFs (platelet-activating factors) (Tan et al. 2010).

Two new fused chromenes, eryvarin V (**49**) and eryvarin W (**50**) (Fig. 8.10), were obtained from the plant roots of *Erythrina variegata*. The isolated chromenes showed very good antibacterial activity against MRSA. Eryvarin W (**50**) demonstrated very good anti-MRSA activity, while eryvarin V (**49**) exhibited only moderate activity (Tanaka et al. 2011). The DCM extract of the stem bark *Paramignya monophylla* exhibited adequate antifungal property against *Cladosporium cladosporioides* strains, as revealed by the thin-layer chromatography bioassay. Six pyranocoumarins, including poncitrin (**51**) and nordentatin (**52**), were isolated from the bark of *P. monophylla*. Structures of these fused natural chromenes were elucidated by spectroscopic methods (Kumar et al. 1995).

Linearly pyranofused coumarin, theraphin D (**53**) (Fig. 8.10), along with other coumarins and xanthones were isolated from the tree (*Kayea assamica*) bark and evaluated for anti-cancer activity against cancerous cells. Compound **53** showed moderate activity against the nasopharyngeal epidermoid carcinoma (KB) cell line. It also exhibited weak anti-malarial activity against the chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *P. falciparum* (Lee et al. 2003).

Soulamarin (**54**) (Fig. 8.10), another naturally occurring chromene, was first isolated from the bark of *Calophyllum soulattri* along with other known compounds.

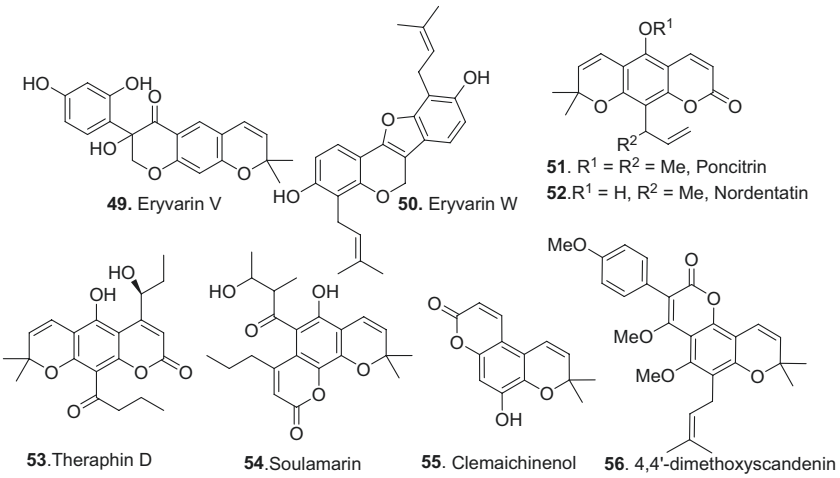


Fig. 8.10 Structures of some important fused naturally occurring chromene derivatives

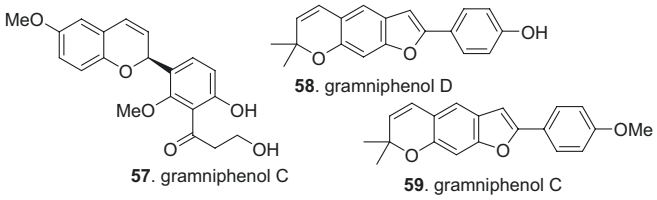


Fig. 8.11 Structures of antiviral gramniphenol C, F and G

The structure of this compound was elucidated using spectroscopic analysis (Ee et al. 2011). Soulamarin showed significant activity against *Trypanosoma cruzi* (Chagas disease). The colorimetric MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide] assay of compound **54** demonstrated that it was active against trypomastigotes, and also possessed trypanocidal property, which has been reported to be mediated by mitochondrial dysfunction, altered permeability of the plasma membrane, leading to the death of *T. cruzi*. Soulamarin may serve as a lead molecule for neglected diseases, particularly Chagas disease (Rea et al. 2013). Clematichinenol (**55**), a pyranocoumarin, was first extracted from the butanol-soluble fraction of aerial parts of *Clematis chinensis* (Shao et al. 1996); 4,4′-dimethoxyscandenin (**56**), a natural chromene derivative, was firstly isolated from *Derris scandens* stems (Rao et al. 1994), and its structure was interpreted by 1D and 2D NMR (nuclear magnetic resonance) (^{13}C, ^{l}H COSY and COLOC) spectroscopic analyses.

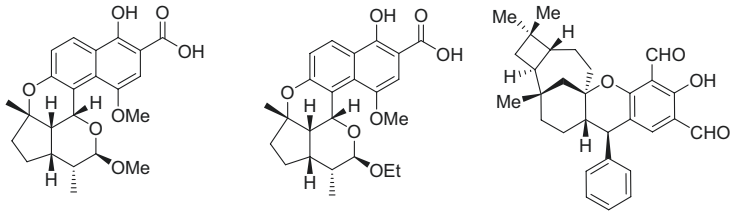
Substituted and fused 2H-chromenes, such as gramniphenol C (**57**), F (**58**) and G (**59**) along with various known and new phenolic compounds, were isolated by Yang and co-workers from the whole plant of *Arundina gramnifolia* of Orchidaceae family (Fig. 8.11). Structures of these isolated compounds have been assigned by 1D

and 2D NMR studies. Isolated chromene compounds along with known phytochemicals were studied for their antiviral activities. Compounds **57**, **58** and **59** showed activity against TMV (tobacco mosaic virus) with IC_{50} values of 21, 41 and 58 μM, respectively. These molecules also displayed very weak anti-proliferative activity against C8166 cells as well as weak anti-HIV-1 activity, as compared to anti-HIV drug azidothymidine (AZT) (Hu et al. 2013).

Abdissa et al. (2016) isolated two highly annulated nepthochromenes, named as parvinaphthol C (**60**) and busseihydroquinone E (**61**), which were isolated from the roots of *P. parvifolia* and *P. bussei* (Family Rubiaceae) roots, respectively (Fig. 8.12). Parvinaphthol C and busseihydroquinone E showed marginal cell inhibitory activity against breast cancer (MDA-MB-231) cells with IC_{50} values of 129.6 μM and 62.6 μM, respectively. (+)-Psiguadial B (**62**) (Fig. 8.12) was isolated by Shao and co-workers from the leaves of *Psidium guajava*, which is widely used in the Chinese medicines (Shao et al. 2010). It shows potent inhibitory activity against HepG2 (human hepatoma) cells with an IC_{50} value of 46 nM. Reisman and co-workers recently synthesized (+)-Psiguadial B through 15 steps of enantioselective process. The synthetic strategy involved the construction of a transfused cyclobutane ring through a Wolff rearrangement/asymmetric ketene addition sequence, followed by a Pd-catalysed C(sp3)–H alkenylation reaction (Chapman et al. 2016).

Recently, Kingston and co-workers isolated seven chromane (**63–69**) (Fig. 8.13) and two novel chromene derivatives (70–71) from the monotypic plant *Koeberlinia spinosa* of the family Koeberliniaceae (Presley et al. 2018). Chromanes **68** and **69** were identified as diastereomeric cyclic derivatives of compound **65** and are thought to have been formed during the separation process. The absolute configuration of isolated chromene molecules at the C-2 chiral centre was assigned as *S* through analysis of the electronic circular dichroism (ECD) spectroscopic data. From among the isolated compounds that were investigated for biological activities, only chroman derivatives **64**, **65**, **68** and **69** exhibited mild anti-plasmodial activity against *P. falciparum* Dd2, while none of them showed any activity against the A2780 ovarian cancer cells.

Flemingia grahamiana is a shrub of the family Leguminosae, found in India. The leaves of this plant are used for the treatment of skin diseases and as a purgative. Gumula et al. isolated three known and nine new chalcone chromene hybrids, namely, flemingins A − C (**72–74**) and flemingins G–O (**75–83**), respectively, from



60. Parvinaphthol C **61**. Busseihydroquinone E **62**. (+)-Psiguadial B

Fig. 8.12 Structures of some highly annulated naturally occurring important anti-cancer chromene derivatives

the leaf extract (DCM–MeOH) of this plant (Fig. 8.14). Structures of these isolated molecules were determined by spectroscopic methods. These molecules were evaluated for their antioxidant and cytotoxic activities. The flemingins A (**72**), B (**73**), C

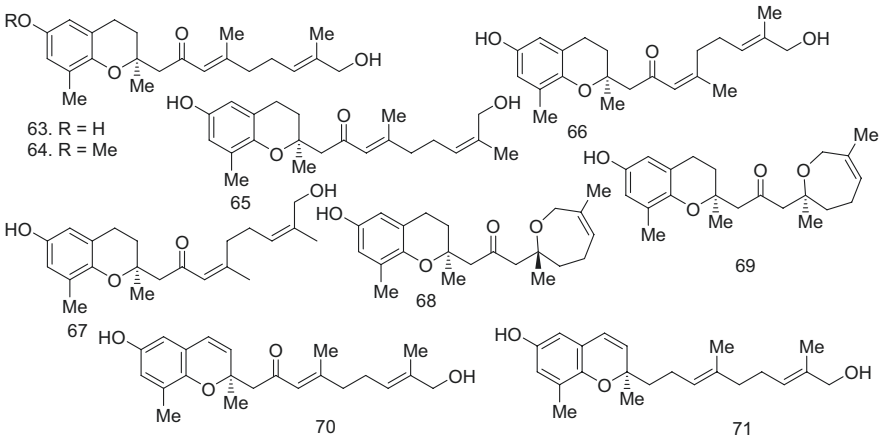


Fig. 8.13 Structures of chromanes and *2H*-chromene derivatives isolated from *Koeberlinia spinosa*

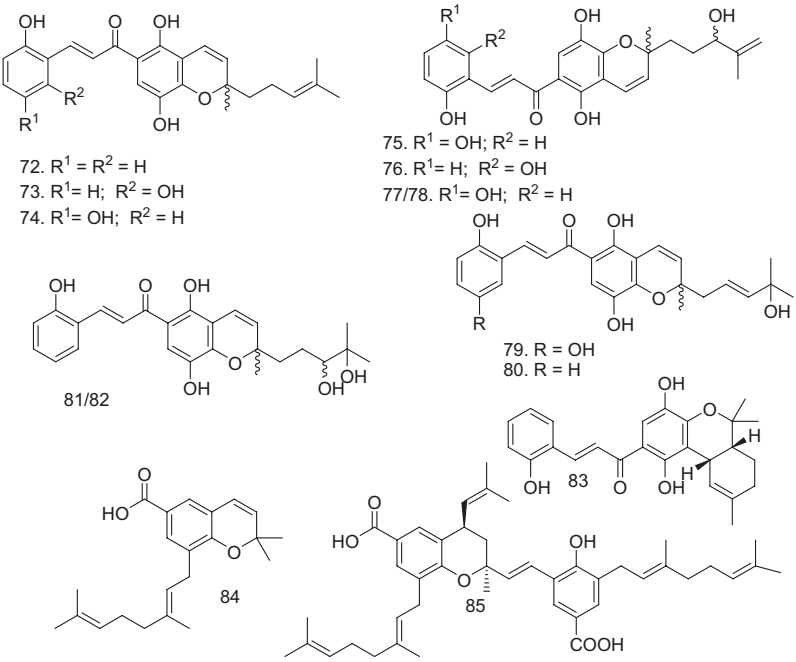


Fig. 8.14 Structures of prenylated *2H*-chromene derivatives isolated from *Piper kelleyi*

(**74**), G (**75**) and H (**76**) were found to possess the DPPH radical scavenging activity (ED_{50} 4.4–8.9 μM), while flemingins A (**72**) and C (**74**) also exhibited anti-proliferative activity against MCF-7 human breast cancer cell lines (IC_{50}, 8.9 and 7.6 μM, respectively). Among all the isolated compounds, from the leaves of this plant, flemingins A displayed the maximum antioxidant activity (ED_{50} = 4.4 μM), while flemingins C exhibited the maximum cytotoxic (IC_{50} 7.6 μM) activity against MCF-7 cell lines (Gumula et al. 2014).

Recently, Jeffrey and co-workers isolated two novel biologically active natural prenylated chromenes (**84** and **85**) from methanolic extract of the leaves of the *Piper kelleyi* plant and determined their structures through 1D and 2D NMR analyses. These isolated chromenes exhibited anti-herbivore activity against a lab colony of *Spodoptera exigua*—a generalist caterpillar. Compounds **84** and **85** reduced the pupal mass and greatly delayed the larval development time, which is an indicator of reduced adult fecundity of the worm (Jeffrey et al. 2014).

Ten new (**86–89**, **94–99**) and four known (**90-93**), biologically active chromene/chromane derivatives were isolated from *Hypericum empetrifolium (*Hypericaceae) (Schmidt et al. 2012) (Fig. 8.15). Compounds **97** and **98** were isolated as a mixture

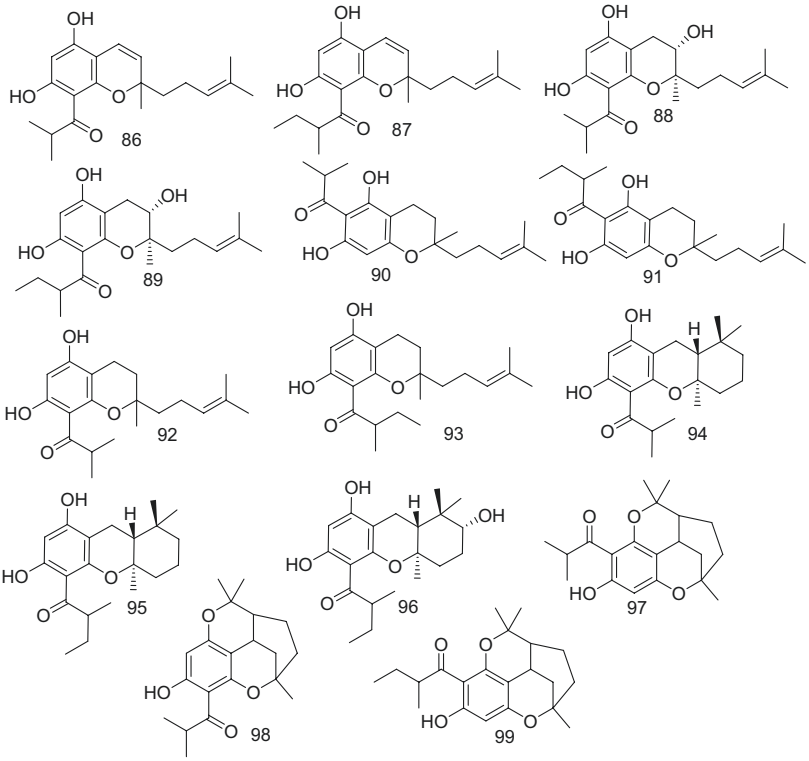


Fig. 8.15 Structures of prenylated *2H*-chromene and chromane derivatives isolated from *Hypericum empetrifolium*

of two inseparable regioisomers. All the isolated compounds exhibited mild antiproliferative activity against human microvascular endothelial cells (HMEC-1). Bicyclic derivatives, empetrikarinens A (**86**) (24.4 ± 2.2) and empetrikarinens B (**87**) (15.0 ± 1.4), and empetrikarinols A (**88**) (23.4 ± 1.0) and empetrikarinols B(**89**) (18.4 ± 1.7), and compounds **90–93** exhibited similar activities (IC_{50} = 29.6 ± 3.5 to 13.3 ± 3.8 μM). Tricyclic analogues empetriferdinan A (**94**), empetriferdinan B (**95**) and empetriferdinol (**96**) also showed weak inhibition against the HMEC-1 cell line. While empetrifranzinan A/B (**97/98**) and empetrifranzinan C (**99**) showed slightly stronger activities, with IC_{50} values of 11.7 ± 1.8 and 9.2 ± 2.0 μM, respectively.

Ren and co-workers isolated fused derivatives of chromene **100**, **101** and **102** along with some other compounds from a $CHCl_3$ soluble extract of the MeOH extract of the dried ground twigs of *Artocarpus rigida* (Ren et al. 2010) (Fig. 8.16). The structure of these molecules was established by NMR spectroscopy data and CD studies. These compounds showed very weak activity against HT-29 human colon cancer cells. Compound **102** exhibited NF-κB inhibitory activity in the NF-κB p50 assay (IC_{50}, 3.7 μM). Cabrera et al. isolated chromeno-flavanone **103** and obovatin **104**, along with other flavanoids from the *n*-hexane extract of *Dalea boliviana* roots (Peralta et al. 2011) (Fig. 8.17). These molecules were evaluated for in vitro tyrosinase inhibitory activity. Compounds **103** and **104** exhibited very low tyrosinase inhibition, even at 100 μM, as compared to Kojic acid.

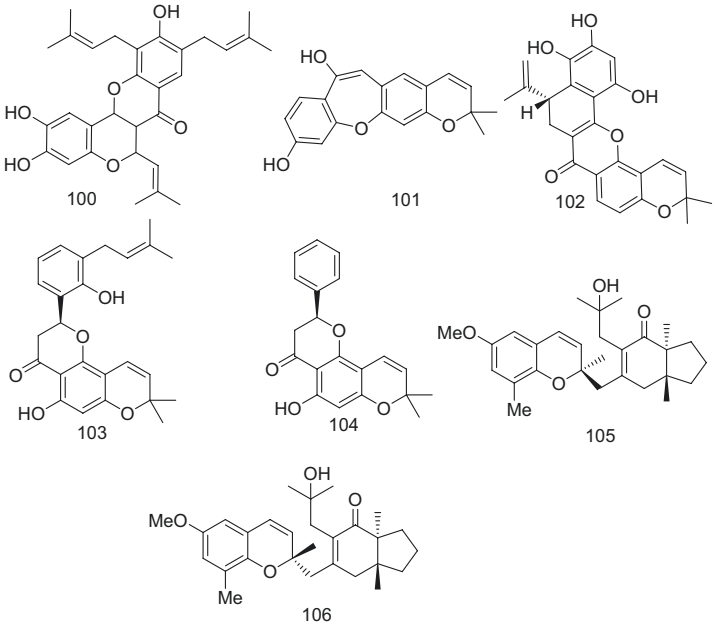


Fig. 8.16 Structures of chromene derivatives isolated from *Artocarpus rigida* and *Cystoseira baccata*

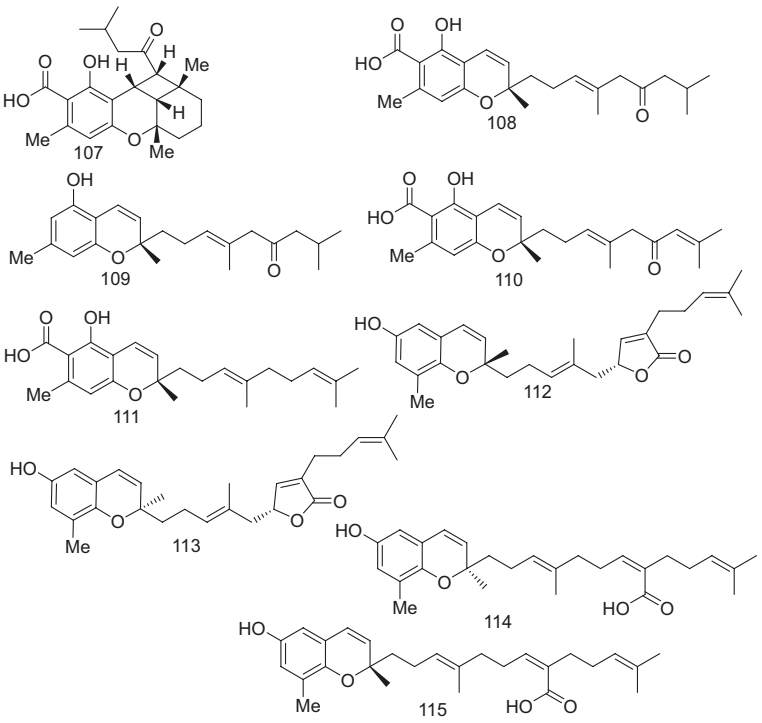


Fig. 8.17 Structures of some important chromene derivatives isolated from the Korean marine tunicate *Botryllus tuberatus*

Two isomeric chromene derivatives **105** and **106** along with a number of meroditerpenoids were isolated from *Cystoseira baccata*- a brown alga (Mokrini et al. 2008). The isolated chromene derivatives were tested for their biological properties. Compound **106** displayed antifouling activity against the growth of macroalgal settlement and microalgae as well as mussel phenoloxidase activity. It was also found to be nontoxic to larvae of oysters and sea urchins.

Four new chromane derivatives, anthopogochromane (**107**), anthopogochromene A (**108**), anthopogochromene B (**109**) and anthopogochromene C (**110**), and one known molecule, daurichromenic acid (**111**), were isolated/obtained from the twigs and leaves of the Chinese medicinal plant, *Rhododendron anthopogonoides* (Iwata and Kitanak 2010). The isolated chromene derivatives were evaluated for anti-inflammatory activity. Chromenes **107** and **108** inhibited compound 48/80-induced histamine release from rat peritoneal mast cells. The inhibitory activity of compound **107** (IC_{50}, 114 μM) and compound **108** (IC_{50}, 63 μM) compared favourably with that of the standard anti-inflammatory drug indomethacin (IC_{50}, 250 μM). However, the other isolated chromene molecules did not show any anti-inflammatory action.

Choi et al. isolated two novel γ-lactone ring bearing chromene derivatives, tuberatolide B (**112**) and 2′-*epi*tuberatolide B (**113**) along with the known compounds, (*R*)-sargachromenol (**114**) and (*S*)-sargachromenol (**115**), from *Botryllus tuberatus*—a Korean marine tunicate (Choi et al. 2011). The structures of these chromene derivatives were established by NMR, MS and CD studies. hFXR transactivation and cytotoxic activities of these molecules were determined by cell-based co-transfection assay. These compounds exhibited potent inhibition of hFXR transactivation without significant effect on the steroid receptors in the transactivation experiments and reduced the binding affinity of the co-activator peptide SRC-1 to hFXR LBD. Tuberatolide B (**112**) strongly inhibited hFXR transactivation (IC_{50}, 1.5 μM) as compared to other derivatives.

8.3 Conclusions

Chromenes, encompassing a large group of biologically important molecules are widely distributed in a variety of different herbs, shrubs and trees, worldwide. A variety of chromene derivatives have been used for thousands of years in different traditional systems of medicine. Various naturally occurring chromene derivatives, such as ageratochromene, alloevodionol, lonchocarpin, millepachine, flemiphilippinones C, busseihydroquinones B − D, β-lapachone, mollugin, wittifurans A–F, theraphin, soulamarin, gramniphenol C–D, (+)-psiguadial B, flemingins A − C, empetrikarinens A, empetrikarinols A, empetriferdinan A, empetriferdinan B, anthopogochromane and anthopogochromene A–C have been found to possess promising pharmaceutical and therapeutic properties. Considering the immense medicinal value of chromene-based phytomolecules and their derivatives, it is only imperative that in the race for discovery of new lead molecules, especially for the treatment of cancer and drug-resistant bacterial diseases, more intensive efforts are warranted for the isolation and screening of novel chromene-based phytocompounds in the search for more potent and less toxic novel therapeutics with unique mechanisms of action.

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Coumarins: An Important Phytochemical with Therapeutic Potential

9

Dilipkumar Pal and Supriyo Saha

Contents

Abstract

Coumarin is a benzopyranone derivative with potential activities. Coumarin is generally obtained from a natural source, and also can be chemically synthesized. Coumarins are abundant in nature, and their sources include plant root, rhizomes, leaves, bark, in addition to several marine plants. Simple coumarins are commonly observed in most of the flowering plants, mainly belonging to the families of *Rutaceae*, *Apiaceae*, *Asteraceae*, *Lamiaceae*, *Clusiaceae*,

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Thymelaeaceae, and *Oleaceae*. In nature, coumarins are of four major subtypes, namely the simple coumarins, pyranocoumarins, furanocoumarins, and the pyrone-substituted coumarins. Osthenol, a prenylated coumarin is a potent monoamine oxidase inhibitor. Coumarins exhibit antifungal, anti-inflammatory activity and antimicrobial activities. Panitins A–G, newer coumarin derivatives are observed with lipopolysaccharide-induced nitric oxide inhibition property, and a new set of coumarins are observed with antiproliferative and anti-leishmanial properties. Oxyprenalated coumarins are observed with melanogenesis modulatory property. Coumarin glycoside shows its potency against diabetic neuropathy. The importance of natural coumarins is correlated to its greater therapeutic potentials with meager toxicity.

Keywords
Coumarin · Antiproliferative activity · Anti-inflammatory activity · Anti-cholinesterase activity · Anti-leishmanial activity

9.1 Introduction

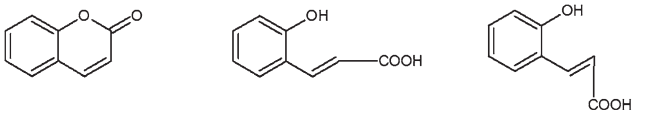
Nature always stands as a golden mark to exemplify the remarkable characteristics of symbiosis. Natural products obtained from plant, minerals, and animal sources have become the basis for the treatment of human ailments. Nowadays, a huge percent of people in developing countries still use herbal medicines that are based largely on different species of plants and animals. The term "coumarin" was first used in the early 1800, which was ideally sourced from the local name of tonka beans, "Coumarou" (Scientific name: *Dipteryx odorata,* belonging to *Fabiaceae* family). Coumarins have a sweet odor, and hence are being used in perfumery industries, since 1882. Also, it is assumed that plants produce coumarins to deter predation, which is one type of a chemical defense mechanisms (Borges et al. 2009; Matos et al. 2015; Stefanachi et al. 2018). The long-known sources of natural coumarin are the tonka beans, white clover (*Trifolium repens*), and woodruff (*Galium odoratum*). Coumarins are abundantly found occurring in different parts of the plants, such as roots, rhizomes, leaves, and bark, and also known to occur in marine plants (Pal and Nayak 2017). Simple coumarins are commonly observed in most of the flowering plants, mainly belonging to the families of *Rutaceae*, *Apiaceae*, *Asteraceae*, *Lamiaceae*, *Clusiaceae*, *Thymelaeaceae*, and *Oleaceae* (Matos et al. 2015). In nature, coumarins are six types, namely the simple coumarins, furanocoumarins, dihydrofuranocoumarin, pyranocoumarin, phenylcoumarins, and bicoumarins (Pal et al. 2018). The simple coumarins, such as 7-hydroxycoumarin and 6,7-dihydroxycoumarin are the hydroxylated, alkylated, and alkoxylated derivatives of the parental compound, coumarin, alongside with their glycosides (Jain and Joshi 2012). Esculetin, osthole, and umbelliferone are simple coumarins; psoralen and bergapten are furanocoumarin; anthogenol and felamidin are dihydrouranocoumarin. Structurally, pyranocoumarins are classified into two types, i.e., linear and

angular, based on the position of methyl group; examples include xanthyletin and inophyllum. Mammea and dispardiol B are the examples of phenylcoumarin, and dicoumarol is an example of bicoumarin (Matos et al. 2015).

Simple coumarins include umbelliferone, esculetin, scopoletin, osthol, and dicoumarol. When furan ring is attached with coumarin molecule in a linear or angular position, the molecule becomes furanocoumarin. Some examples include psoralen, bergapten, methoxsalen, and paradisin A and C. They are most commonly found in *Rutaceae* and *Apiaceae* family members. When a six-membered oxygen-containing heterocyclic ring is attached with a basic coumarin nucleus in an angular or linear position, the molecule is known as pyranocoumarin (anomalin). Finally, a six-membered oxygen-containing heterocyclic ring with exocyclic double bond at second or fourth position, when fused with coumarin structure molecule is called as pyronocoumarin (Singh and Pathak 2016). In the year of 1948, named Dr. Karl Paul Link first developed a synthetic coumarin named as "Warfarin" as a rodenticide. Since from 1954, it is openly used by humans. The name warfarin comes from WARF (Wisconsin Alumni Research Foundation) and ARIN (last four letters from eight letters coumarin). After that, warfarin was identified as first oral thrombin inhibitor, and hugely accepted as anticoagulant agent (Pirmohamed 2006). In the recent times, the fruits, stems, and barks of *Calophyllum dispar* (commonly known as Alexandrian laurel balltree) are used as a source of coumarins that exhibit cell toxic behavior (Guilet et al. 2001); also, lots of microbial sources like *Escherichia coli* and *Streptomyces coelicolor* are being explored to develop different coumarin derivatives and coumarin antibiotics (e.g., novobiocin, coumermycin, and clorobiocin) (Heide 2009).

9.2 Structural Features of Coumarin

Coumarin is chemically known as 1,2-benzopyrone/2H-chromen-2-one with the molecular formula, $C_9H_6O_2$ and it has a molecular weight of 146.14 g/mol. Coumarin (ortho-hydroxycinnamic acid) is observed with soothing vanilla smell and traditional anticoagulation property. The appearance of coumarin was shaped with colorless to white needle/powder like texture and sour taste (National Center for Biotechnology Information n.d.). In the structure of coumarin, there is no hydroxyl group, and hence it is tough to dissolve in water. To overcome this problem, the structure is generally heated or kept in water for certain time until it is dissolved to give a yellowish color. This reaction ideally forms the basic salt of coumarinic acid. When this salty solution is treated with acid or carbon dioxide gas, it turns into coumarin (Fig. 9.1). Coumarinic acid is the characteristic feature of coumarin formation, which is obtained by the ring opening reaction of pyrone (Sethna and Shah 1945). The double bond at C-3 position of coumarin makes the molecule to behave like an auxochrome, which is used as a fluorescent dye in the textile industry. Coumarins are obtained from synthetic as well as natural sources like plants and marine fungi. Here, the discussion is made strictly adhering to the recent development of coumarin derivatives obtained from natural and marine sources.



Coumarin Coumarinic acid Coumaric acid

Fig. 9.1 Structures of coumarin, coumarinic acid, and coumaric acid

9.3 Sources and Therapeutic Applications of Coumarin

Coumarins are being identified in various medicinal plants, such as *A. pubescens*, *F. trachycarpa* Boiss, *A. dahurica*, *F. narthex* Boiss., *F. sinkiangensis*, *F. gummosa*, *N. incisum*, *Zosima absinthifolia*, belonging to *Apiaceae* family; *Murraya paniculata*, *Paramignya trimera*, *Micromelum minutum*, belonging to *Rutaceae* family; *Sapium baccatum*, *Trigonostemon lutescens,* belonging to *Euphorbiaceae* family; *Calophyllum brasiliense*, *Calophyllum inophyllum*, belonging to *Calophyllaceae* family; *Streblus indicus*, belonging to *Moraceae* family; *Corydalis heterocarpa*, belonging to *Papaveraceae* family; *Juglans mandshurica* Maxim., belonging to *Juglandaceae* family; *Polygala boliviensis*, belonging to *Polygalaceae* family; and *Rhizophora mucronata*, belonging to *Rhizophoraceae* family. These plants provide various health benefits being an anti-rheumatic, analgesic, anti-inflammatory, anticancer, antioxidant, antipyretic, antiseptic, and a disinfectant, and used for treatment of anorexia, arthritis, urinary tract infection, and stomach pain. Importantly, *Apiaceae* and *Rutaceae* family members are the main source of natural coumarins.

9.3.1 Coumarins Isolated from Apiaceae Family

Baek et al. (2019) isolated osthenol (Fig. 9.2), a prenylated coumarin from dried roots of *Angelica pubescens.* The plant was basically found in Japan and China, and belongs to *Apiaceae* family. It is known for anti-inflammatory, antibacterial, cytotoxic, and vasodilator activities. In a study, osthenol was tested against recombinant human monoamine oxidase enzyme (monoamine oxidase A and B) along with other phenylpropane coumarin and furanocoumarin derivatives (bakuchicin, psoralen, isopsoralen, scopoletin, and isoimperatorin) isolated from *Psoralea corylifolia*, *Anthriscus Sylvestris*, *and A. dahurica*. The results revealed that osthenol inhibits human recombinant monoamine oxidase A and B with greater selectivity toward human recombinant monoamine oxidase A enzyme. These data stated the importance of osthenol as a monoamine oxidase inhibitor (Baek et al. 2019).

Dikpinar et al. (2018) isolated and evaluated four pure coumarin derivatives (Fig. 9.3), namely crenulatin (6-formyl-7-methoxycoumarin), suberosin (7-methoxy-6-prenylcoumarin), marmesin senecioate [((−)-prantschimgin) as dihydrofuranocoumarin derivative], and ulopterol [6-(2′,3′-dihydroxy-3′-methylbutyl)-7-methoxy-coumarin] from the rhizomes of *F. trachycarpa* Boiss. using *n*-hexane, dichloromethane and

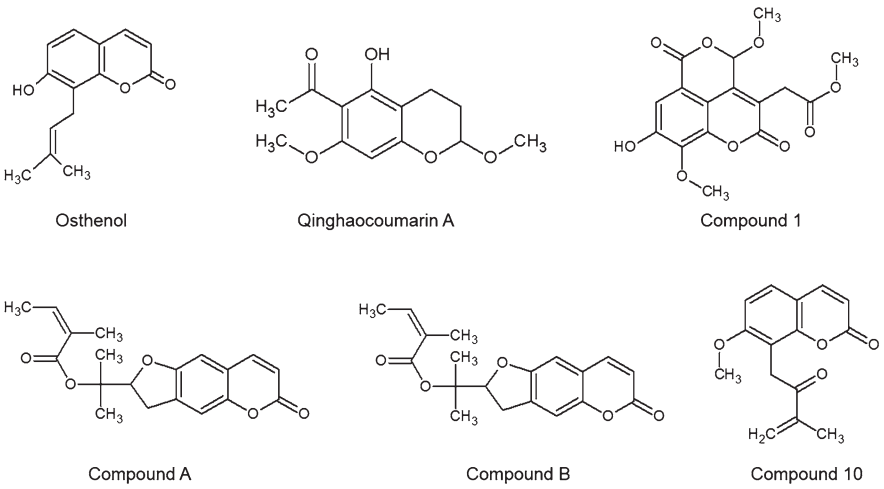
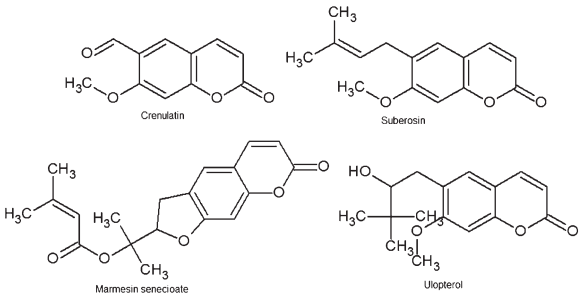


Fig. 9.2 Structures of osthenol, qinghaocoumarin A, compound 1, compounds A and B, and compound 10 (most effective)

Fig. 9.3 Structures of coumarins isolated from *Ferulago trachycarpa* Boiss



methanol as solvents, and evaluated their antimicrobial efficacy against *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus feacalis*, along with fungal strains, *Candida albicans*, *C. tropicalis*, and *C. parapsilosis*. Compounds 1, 2, and 3 were isolated from *n*-hexane extracts, followed by fractionation using dichloromethane and methanol, chloroform, and methanol as liquid for eluents and purified by thin-layer chromatography using (toluene, dichloroform, ethyl acetate, acetonitrile) and (benzene, ethyl acetate) as mobile phase; compound 4 was extracted from dichloromethane and purified by toluene, chloroform, ethyl acetate, and acetonitrile as mobile phase. The outcomes revealed that *Candida albicans* was inhibited by dichloromethane and methanolic extract and *n*-hexane fraction greatly worked against *S. aureus*, but the pure isolated compounds did not exhibit any antimicrobial activity (Dikpinar et al. 2018).

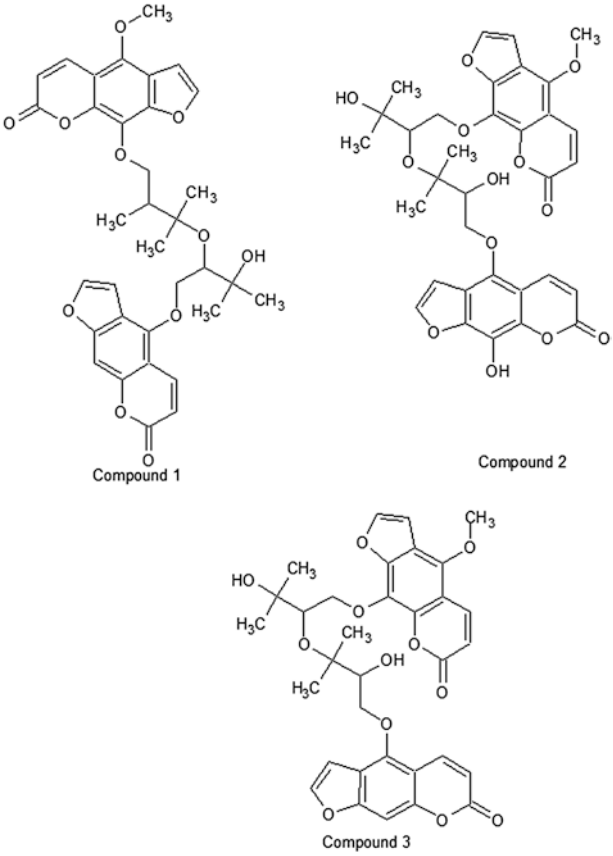


Fig. 9.4 Furanocoumarins isolated from *Angelica dahurica*

Bai et al. (2016) isolated three new furanocoumarins (Fig. 9.4) along with (previously established) coumarin from the roots of *Angelica dahurica*. The isolation of the 23 compounds were done by partitioning with petroleum ether, ethyl ethanoate, and *n*-butanol as solvents; compounds were isolated using different fractions of petroleum ether, acetone, methanol, and water. The structural mass was identified by ultra-performance liquid chromatographic technique using water, methanol, and formic acid as mobile phase. Diphenyl picrylhydrazyl and azino-bis-ethylbenzothiazoline sulfonic acid methods were used to determine reducing power; the antiproliferative property of the compounds was analyzed against HeLa (cervical carcinoma), HepG2 (human liver carcinoma), and MCF-7 (breast cancer) cell lines. The outcomes revealed that among the 23 compounds, compounds 5, 16, 17, 18, and 19 were observed with slight inhibition of free radical generation and compound 16 showed good scavenging of free radical generation as per diphenyl picrylhydrazyl and azino-bis-ethylbenzothiazoline sulfonic acid methods, respectively. Compounds 4, 6, and 19 showed inhibition of growth for HeLa (cervical carcinoma)

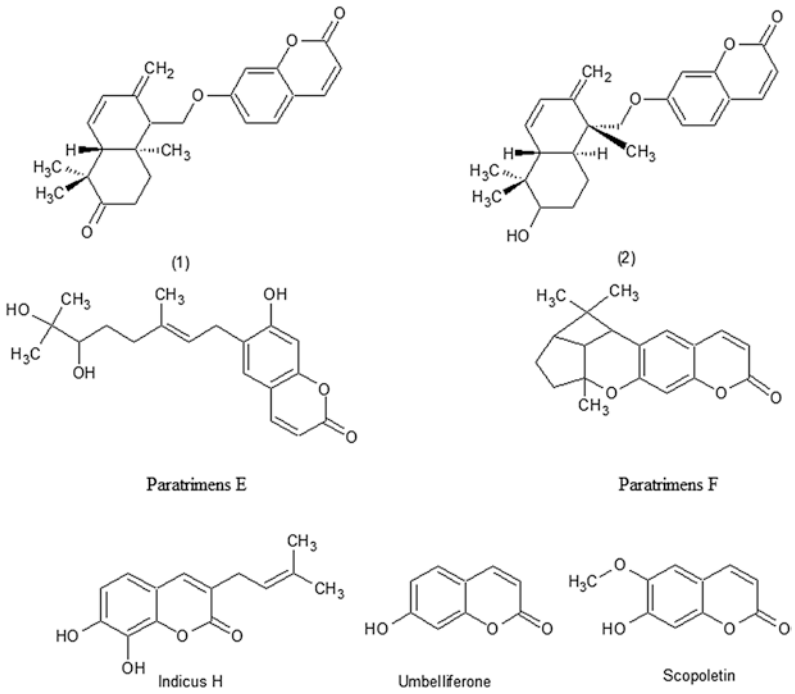


Fig. 9.5 Structure of components isolated from *Ferula narthex* Boiss., *Paramignya trimera*, and *Streblus indicus* (Bur)

and HepG2 (human liver carcinoma) cell lines. These data stated the importance of coumarins isolated from *Angelica dahurica* to inhibit the generation and progression of cancer (Bai et al. 2016).

Bashir et al. (2014) isolated and evaluated two new sesquiterpene coumarins (1 and 2), and three previously generated coumarins, such as conferol, conferone, and umbelliferone (Fig. 9.5) from *Ferula narthex* Boiss, by fractionation of methanolic extract of the plant using *n*-hexane, *n*-butanol, chloroform, and ethyl ethanoate as solvents followed by sub-fractionation using ethyl ethanoate, *n*-hexane, and acetone as eluents; then all the compounds except umbelliferone were evaluated against *Leshmania donovani* to check the life cycle arrest. The outcomes revealed that conferol observed with maximum anti-leishmanial activity (Bashir et al. 2014).

Li et al. (2015) isolated nine sesquiterpene coumarins (one is novel named as sinkiangenorin D (Fig. 9.6), followed by ten previously established ones, named as lehmannolol, lehmannolone, episamarcandin, colladonin, sinkianone, fekrynol, fekolone, feselol, umbelliprenin, and farnesiferol C) from the seeds of *Ferula sinkiangensis*, and evaluated their cell toxic nature against HeLa (cervical carcinoma), K562 (myelogenous leukemia), and AGS (stomach adenocarcinoma) cell lines. The isolation of the molecules was done by extraction of the seeds by ethanol

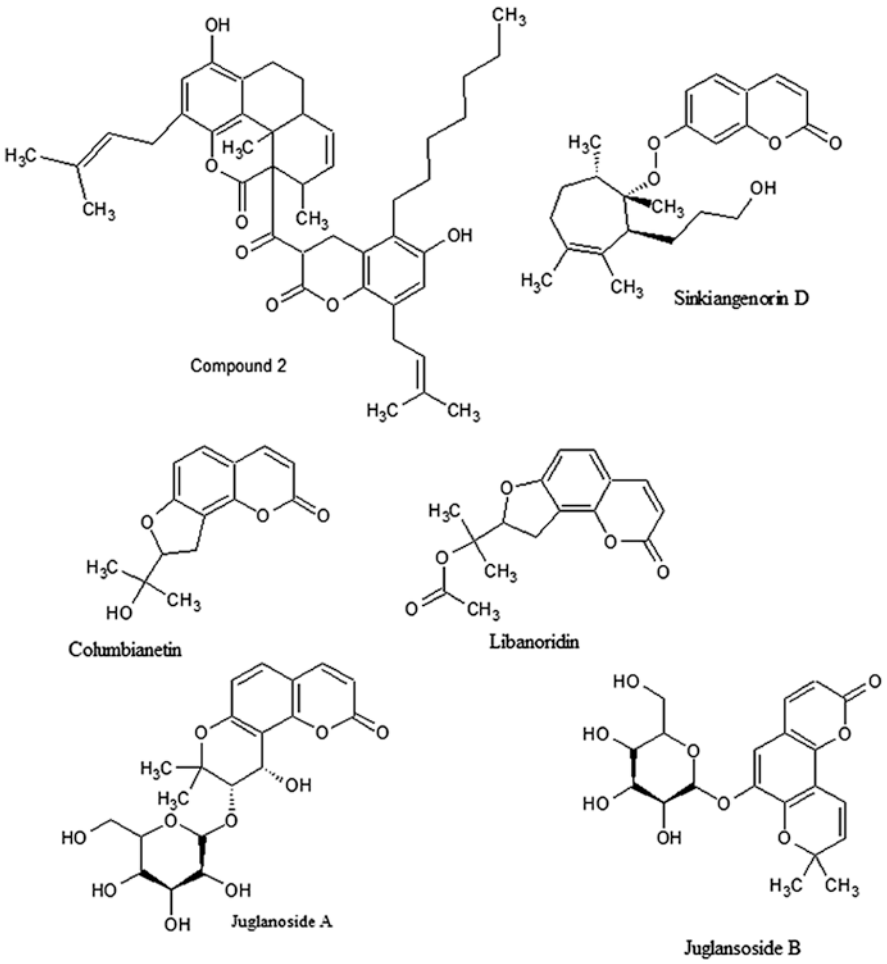


Fig. 9.6 Structure of coumarins from *Corydalis heterocarpa*, *Ferula sinkiangensis*, *Calophyllum inophyllum*, and *Juglans mandshurica* Maxim

followed by partitioning with petroleum ether and dichloromethane, and then the dichloromethane layer was further fractioned using chloroform, methanol, and water as solvents. The outcomes revealed that HeLa (cervical carcinoma) and K562 (myelogenous leukemia) were moderately inhibited by sinkiangenorin D, whereas umbelliprenin inhibited the growth of AGS (stomach adenocarcinoma) cell lines. These data confirmed the cell toxicity behavior of isolated coumarins (Li et al. 2015).

Iranshahi et al. (2014) isolated three drimane-type sesquiterpene coumarin (conferone, mogoltacin, and feselol) from fruits of *Ferula gummosa* (Fig. 9.7) using dichloromethane as solvent, followed by partitioning using petroleum ether and variable

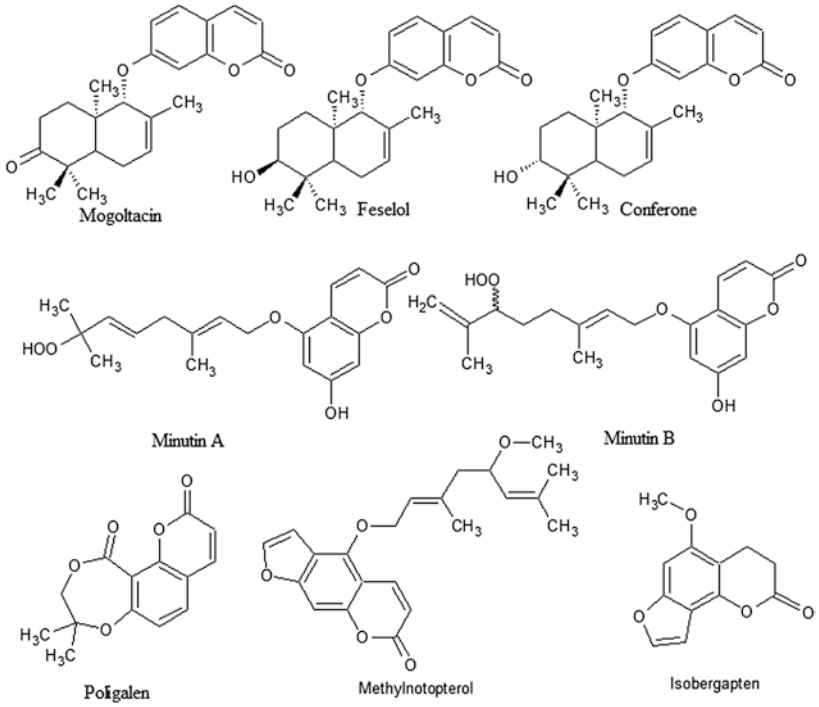


Fig. 9.7 Structure of coumarins isolated from *Ferula gummosa*, *Micromelum minutum*, and Notopterygium incisum

concentrations of acetone, and then the coumarins and control verapamil were evaluated against resistant MCF7/doxorubicin cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay method. The outcomes revealed that fesolol, mogaltacin, and conferone increased the uptake of doxorubicin in doxorubicin-resistant MCF7 breast cancer cell line (Iranshahi et al. 2014).

Seo et al. (2013) isolated ten different coumarins, named as isoimperatorin, imperatorin, senbyakangelicol, oxypeucedanin, byakangelicol, t-OMe-oxypeucedanin hydrate, t-OMe-byakangelicin, angelol H, byakangelicin, and oxypeucedanin hydrate from the roots of *Angelica dahurica* plants by extraction with chloroform, absolute ethanol and ethanol (50%), and water as solvents, and then the acetylcholinesterase inhibitory activity was evaluated against human acetylcholinesterase (hChE) and butylcholinesterase (bChE) enzymes by colorimetric process. The outcomes showed that ethanolic extract of the plant (100 μg/ml) inhibited the progression of enzymatic reaction with half maximal inhibitory concentration (IC_{50}) of 26.40 and 14.70 μg/ml, respectively. Furhter, compounds like byakangelicol, t-OMe-byakangelicin, and byakangelicin were also effective in inhibiting these enzymes. These data clearly state the importance of these coumarins against cholinesterase enzyme (Seo et al. 2013).

Zheng et al. (2018) isolated and evaluated six new coumarins notoptetherins A−F, along with notoptol, methylnotoptol, anhydronotoptol, bergamottin, notopterol, methylnotopterol, ethylnotopterol, isoimperatorin, bergapten, imperatorin, isobergapten, pimpinellin, nodakenetin, nodakenin, (S)-angenomalin, seselin, aurapten, 7-O-prenylumbeliferone, scopoletin (Fig. 9.5), and (S)-6-O-methylscorzocreticin from roots and rhizomes of *N. incisum* using ethanolic extract, followed by fractionation with petroleum ether, chloroform, and *n*-butanol and sub-fractionation using different ratios of petroleum ether and ethyl ethanoate, petroleum ether and acetone, methanol and water, and acetonitrile and water as solvent system. They were evaluated against lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. The outcomes revealed that methylnotopterol and isobergapten (Fig. 9.7) greatly inhibited the production of nitric oxide (Zheng et al. 2018).

Bahadir et al. (2011) isolated two coumarin derivatives (compounds A and B) (Fig. 9.2) from *Z. absinthifolia* using *n*-hexane as solvent. Then, both the compounds and *Z. absinthifolia* extract were treated to carbon tetrachloride-induced rats, and assessed the inhibition of aspartate aminotransferase and alanine aminotransferase enzymes. Also, anti-inflammatory activity was evaluated by the inhibition of tumor necrosis factor generation on lipopolysaccharide-induced human monocytic leukemia cell line using dexamethasone and aspirin as standards. The outcomes revealed that both isolated coumarins and extract showed good effects of degeneration and induction of hepatocytic apoptosis with very minimal sense of bridging necrosis. The minimization of inflammation was greater with 50 μg/ml of extracts; both compounds showed very minimal activity. These data indicate that if inflammation was treated with both coumarins and extract, it will provide good results (Bahadir et al. 2011).

9.3.2 Coumarins Isolated from Rutaceae Family

Wang et al. (2019) isolated seven newer coumarins (panitins A–G) from the fractionation of ethanolic extracts of *Murraya paniculata* plant using petroleum ether and chloroform as solvents; here panitin A was a yellow-colored amorphous powder, panitins B–G were in specific size and all the molecules had its characteristic molecular features. A total of 42 compounds were isolated; among them, pantin D, *trans*-dehydroosthol, and exotimarin-I showed maximum inhibition against lipopolysaccharide-induced nitric oxide generation (Wang et al. 2019).

Dang et al. (2017) isolated and evaluated two acridones, paratrimerins C and paratrimerins D, and two new coumarins, paratrimerins E and paratrimerins F (Fig. 9.5) from the roots of *Paramignya trimera* to identify anti-α-glucosidase inhibitory activity, considering acarbose as the standard molecule. Mainly, 16 molecules, such as paratrimerins C–F, ostruthin, umbelliferone, scopoletin, ninhvanin, xanthyletin, pandanusin A, citrusinine-I, glycocitrine-III, oriciacridone E, 5-hydroxynoracronycin, aedalin A, and vanillic acid were isolated from the methanolic extract of the plant. The outcomes revealed that 5-hydroxynoracronycin and

ostruthin effectively inhibited α-glucosidase enzyme. These data indicate the importance of coumarins as an antidiabetic agent (Dang et al. 2017).

Sakunpak et al. (2013) isolated six coumarins [minutin A and B (newly isolated) (Fig. 9.7), 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, 8-hydroxyisocapnolactone-2′,3′-diol, 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, and clauslactone E (previously identified)] from the leaves of *M. minutum* by stepwise gradient method using column chromatographic technique, involving *n*-hexane, ethyl ethanoate, and methanol solvents. Minutin A and B were generated from reverse-phase chromatography using methanol and water with a ratio of 65:35; 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol was obtained from acetone and water with 38:62 ratio, whereas compounds 4, 5, and 6 were generated from acetone and water with 40:60 ratio. The anti-leishmanial activity against promastigotes, considering amphotericin as standard, and anticancer activity against lung cancer cell lines (lung adenocarcinoma cell lines A549, SBC3, K562, and K562/ADM), considering doxorubicin as standard were evaluated. The outcomes revealed that antileshmanial activity was mainly observed with minutin A, minutin B, clauslactone E, 8-hydroxyisocapnolactone-2′,3′-diol, 8,4″-Dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol and 8-Hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol against SBC3 A549 K562 K562/ADM cell lines with IC_{50} (μM) of 23.7, 18.2, 13.2, 9.5; 9.6, 17.5, 8.7, 6.7; 3.7, 10.4, 12.1, 10.8; 8.8, 10.1, 16.9, 10.1; 92.6, 212.9, not active, 246.6; not active, not active, not active, 97.50 as compare to standard doxorubicin less than 0.4, 0.68, 0.68, greater than 5.5, respectively (Sakunpak et al. 2013).

9.3.3 Coumarins Isolated from Euphorbiaceae Family

Li et al. (2019a, b) isolated and evaluated seven coumarins along with one monoterpenoid galloylglycoside from the stem bark of *Sapium baccatum*. The isolation was done by ethyl acetate and petroleum ether as solvents; the solvents were observed with 31 compounds, and among them, 23 compounds were already established, whereas eight compounds were newly isolated. Enantiomeric compounds (1, 2, and 7) were identified by high-performance liquid chromatography with chiral feature, and electronic circular dichroism techniques. The outcomes revealed that compound 1 (Fig. 9.2) was observed with greater inhibition of tumor necrosis factor-α as compared to quercetin as standard with greater inhibition against *Candida albicans* fungal strain (Li et al. 2019a, b).

Yang et al. (2019) claimed the isolation of a series of furanocoumarins from the twigs of *Trigonostemon lutescens*, which showed inhibition against HCT116 (human colorectal carcinoma), HeLa (cervical carcinoma), and HepG2 (human liver carcinoma) cell lines. The isolation process was performed by collecting four main isolates using petroleum ether, ethyl ethanoate, and *n*-butanol as solvents; then, the ethanoate extract was further fractioned using petroleum ether and ethyl ethanoate (25:(1–0.1) ratio, which mainly isolated fraction F; fraction F was fractioned further using petroleum ether and ethyl ethanoate (20:(1–0.1)), which

resulted in the production of fraction F2, which was again fractioned by petroleum ether and ethyl ethanoate (30:1–10:1) to isolate fraction F2.2, and further fractioned using methanol and water with 2:1 ratio to isolate fraction F2.2.1, and then fractioned using the same diluent with 58:42 ratio which produced compound 2; fraction F2.2.4 was fractioned to compound 1 using methanol and water (47:52). Fraction F3 was fractioned using methanol and water with 30:70 ratio, and further eluted with petroleum ether and ethyl ethanoate with 20:1–10:1 ratio to obtain fraction F3.2, which was further fractioned using dichloromethane and methanol (2:1) and eluted by semi-preparative chromatography to get compounds 9, 6, and 3. Compounds 5, 8, 10 (Fig. 9.7) were obtained from fraction F 3.2.1 using methanol and water (40:60) as solvent system and compound 4 was isolated from fraction F 3.2.3 using methanol and water (58:42) solvent mixture. Rapid Resolution Liquid Chromatography (RRLC) system coupled with Agilent 6520 Accurate-Mass Q-TOF Mass Spectrometer (Agilent Ltd.) with positive and negative electrospray modes technique was used to satisfy the structure of compounds. The cytotoxicity assay was performed for total extract, petroleum extract, ethyl ethanoate extract, *n*-butanol, fraction F and F1 against mentioned cell lines, which confirmed that only compound 10 showed remarkable inhibition against all the cell lines (Yang et al. 2019).

9.3.4 Coumarins Isolated from Calophyllaceae Family

Hernandez et al. (2019) isolated three mammea-type coumarin mammea (A/BA, A/BB, A/BD) from *Calophyllum brasiliense* using *n*-hexane and ethylacetate (95:5 ratio) solvent, and evaluated the growth inhibition of *Trypanosoma cruzi*. The outcomes showed that coumarin-type mammea A/BA and mixture of coumarins exhibited greater inhibition against trypanocides with observable morphological alteration and growth cessation (Hernandez et al. 2019).

Li et al. (2016) isolated six coumarins from the leaves of *Calophyllum inophyllum* by extraction, fractionation, and sub-fractionation techniques using ethanol; petroleum ether, chloroform, ethyl ethanoate, *n*-butanol; and methanol and water, respectively, as solvents, and among them, four were newly identified and named as (−)-12-methoxyinophyllum A, (+)-12-methoxyinophyllum H-1, (−)-12-methoxyinophyllum H-2, and inophyllum J (Fig. 9.8), and two previously identified coumarins were 12-ethoxyinophyllum D and isoinophynone. The mass spectroscopic data suggested that molecular formula of (−)-12-methoxyinophyllum A (colorless liquid) is $C_{26}H_{26}O_5$ (molecular weight calculated: 441.1672), molecular formula of (+)-12-methoxyinophyllum H-1 (colorless needle) is $C_{26}H_{26}O_5$ (molecular weight calculated: 441.1669), molecular formula of (−)-12-methoxyinophyllum H-2 (colorless needle) is $C_{26}H_{26}O_5$ (molecular weight calculated: 441.1670), and inophyllum J existed in two isomeric forms with molecular formula $C_{25}H_{22}O_5$ (molecular weight calculated: 425.1355). These data along with ^{1}H and ^{13}C NMR data convinced the isolation of coumarins from *Calophyllum inophyllum* (Li et al. 2016).

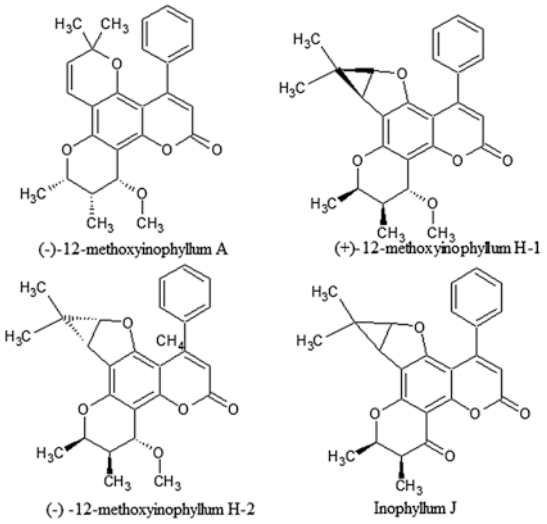


Fig. 9.8 Structure of four new coumarins isolated from *Calophyllum inophyllum*

9.3.5 Coumarins Isolated from Moraceae Family

He et al. (2017) isolated new coumarin and benzofuran glycosides from *Streblus indicus*, which are found to have cytotoxic behavior against human bladder carcinoma cell line (EJ) and human hepatocellular carcinoma cell line (SMMC-7721). The isolation of the compounds was done from the aqueous ethanolic extract of the plant followed by fractionation by petroleum ether, ethyl ethanoate, and *n*-butanol and finally sub-fractioned using different ratios of methanol and water. A total of 19 molecules were isolated, which include indicus (A–H), methylpicraquassioside A, bergapten, bergaptol-O-β-D-glucopyranoside, bergaptol-5-O-α-L-rhamnopyranosyl-(1/6)-β-D-glucopyranoside, (S)-marmesin, (S)-marmesinin, (2S,3R)-3-hydroxynodakenin, (S)-columbianadiratimetin G, umbelliferone, scopoletin, and scoparone. Among them, indicus H, umbelliferone, and scopoletin (Fig. 9.5) showed good inhibition against cell lines (He et al. 2017).

9.3.6 Coumarins Isolated from Papaveraceae Family

Kang et al. 2009 isolated and evaluated two coumarins, columbianetin and libanoridin (Fig. 9.6) from the plant *Corydalis heterocarpa* against lipopolysaccharide-induced human colon carcinoma cells (HT-29), using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay method. The isolation of the molecules was done by extracting with methanol and dichloromethane followed by fractionation with *n*-hexane, aqueous methanol, *n*-butanol, and water as solvents. The outcomes showed that inhibition of generation

of inflammatory mediators (cyclooxygenase-2, tumor necrosis factor-α, interleukin) and greater cell cycle arrest for the progression of human colon carcinoma cells were promoted by libanoridin, not by columbianetin. These data indicate the importance of libanoridin over columbianetin to conquer against colon cancer progression (Kang et al. 2009).

9.3.7 Coumarins Isolated from Juglandaceae Family

Lou et al. (2018) identified the apoptosis-inducing property of seven coumarins isolated from the bark of *Juglans mandshurica Maxim* plant; among them, juglansoside A and B (Fig. 9.6) were the two new coumarins and five coumarins were previously identified. The isolation of coumarins was done by fractionation of ethanolic extract of the plant using ethyl ethanoate and *n*-butanol as solvents, and then the eluents were sub-fractioned using different ratios of methanol and water. The molecules were then evaluated for apoptosis-inducing property against human hepatocellular carcinoma (HepG2 and Hep3B) cell lines. The outcomes revealed that juglansoside A and B effectively inhibited the progression of HepG2 and Hep3B cell lines with IC_{50} value of 61.07 and 64.46 μM, respectively, whereas other molecules caused effective induction of programmed cell death. These data confirm the anticancer activity of the isolated coumarins against human hepatocellular carcinoma (Lou et al. 2018).

9.3.8 Coumarins Isolated from Polygalaceae Family

Silva et al. (2016) isolated and evaluated a new coumarin named as poligalen (Fig. 9.7) from the aerial parts and roots of *Polygala boliviensis* (the extraction started from the methanolic extract of the plants followed by fractionation using *n*-hexane, ethyl ethanoate, and chloroform) and the release of tumor necrosis factor and interleukin was determined by enzyme-linked immunosorbent assay using DuoSet kit, as well as the RAW 264.7 macrophage was used to analyze the effects of poligalen on luciferase-nuclear factor kappa. The outcomes revealed that poligalen reduced the release of tumor necrosis factor and interleukin. Further, it did not caused any change in the cell structure, and increased the kappa factor in RAW 264.7 cells (Silva et al. 2016).

9.3.9 Coumarins Isolated from Rhizophoraceae Family

Taniguchi et al. (2018) isolated and evaluated two new coumarins, one new xanthone and 14 previously established compounds, such as methoxyinophyllum P, calocoumarin B, benzoic acid, amentoflavone, naringenin, calophyllolide, brasimarin C, 6-deoxy-jacareubin, inophyllum C, isocalophyllic acid, inophyllum E, calophyllic acid, jacareubin, and 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)xanthone

from the methanolic extract of leaves of *Rhizophora mucronata* against human acute promyelocytic leukemia (HL60) and human cervical cancer (HeLa) cell lines. The outcomes revealed that 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)xanthone greatly inhibited the cervical carcinoma (IC_{50} = 4.8 μM) and leukemia cell lines (IC_{50} = 1.0 μM) followed by methoxyinophyllum P, amentoflavone, and brasimarin C in the process of inhibition of cancer progression (Taniguchi et al. 2018).

9.3.10 Coumarins Isolated from Marine Sources

Li et al. (2019a, b) isolated coumarin and lignan derivatives from *Artemisia annua*, and evaluated their antifungal activity against *Fusarium oxysporum*, *F. solani*, and *Cylindrocarpon destrutans*. The isolation was performed using ethanolic extract of the plant followed by preparative thin-layer chromatography using (petroleum ether: acetone with 3:1 ratio) and purification was performed using semi-preparative high-performance liquid chromatographic technique. A total of 16 compounds were isolated from the process, and among them, compound 1 (racemic qinghaocoumarin A) (Fig. 9.2) showed maximum inhibition against all the fungal strains with minimal inhibitory concentration of 18.75, 18.75, and 25.00 μg/ml. These data confirmed the inhibition of fungal growth by racemic coumarin qinghaocoumarin A (Li et al. 2019a, b).

Kamauchi et al. 2018 isolated five coumarin derivatives as compounds 1 and 2 (Fig. 9.6) were the unusual tetracyclic coumarin and compounds 3–5 were dimer of coumarin. The isolation of coumarins was achieved when chloroform extract of marine fungus *Eurotium rubrum* was treated with diethyl 1,3-acetonedicarboxylate and piperidine, where compounds 1–3 were collected as a yellow powder and compounds 4–5 were collected as a yellow oil. Finally, all the molecules were evaluated against inhibition of tyrosinse enzyme. The outcomes revealed that compound 2 (Fig. 9.5) exhibited greater inhibition of tyrosinase enzyme (IC_{50} value = 1.2 μM). These facts state the importance of isolated coumarins for inhibition of tyrosinase enzyme (Kamauchi et al. (2018).

9.4 Conclusions and Future Scope

This chapter provides a detailed knowledge about the chemistry and applicability of natural coumarins. Coumarin was obtained from naturally abundant plant families, including *Apiaceae* and *Rutaceae*. Various examples of their role in health benefits are stacked in a row, such as osthenol and a prenylated coumarin showing potent monoamine oxidase inhibition; coumarins from *A. annua* showing antifungal activity; coumarins from *S. baccatum* and *C. heterocarpa* observed with anti-inflammatory activity; coumarins from rhizomes of *F. trachycarpa* Boiss showing antimicrobial activity; panitins A–G, newer coumarin derivatives, obtained from *M. paniculata*, and notoptetherins A − F produced from the roots and rhizomes of *N. incisum* observed with lipopolysaccharide-induced nitric oxide inhibition on RAW

264.7 macrophages; new coumarin derivative from the roots of *A. dahurica*, from the bark of *S. indicus*, and from *J. mandshurica Maxim* showing antiproliferative activity, sesquiterpene coumarins from *F. narthex*, from seeds of *F. sinkiangensis*, and monoterpene coumarin from *M. minutum* leaves observed with anti-leishmanial activity; oxyprenalated coumarin with melanogenesis modulatory activity; coumarin glycoside from *H. paniculata* observed with anti-diabetic neuropathy; and coumarin from roots of *A. dahurica* showing anti-cholinesterase activity. So, this chapter provides a collective knowledge for the scientists to use natural coumarins in an effective way. In future, due to antioxidative, antimicrobial, and anti-inflammatory properties of coumarin, it can be used as a sun-blocking agent, anti-ageing agent, anti-acne agent in cosmetology, and for maintaining oral hygiene, to treat brain-ageing behavior in Alzheimer's disease, as well as it acts as an anticholinergic agent toward Parkinsonism. Nowadays, packaged food is one of the booming industries; here, coumarin also plays an important role to preserve the content with pleasant smell, and by exhibiting antimicrobial property. This applicability of coumarin attracts scientists to use their expertise in the way for the betterment of mankind.

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Pomegranate Peel and Its Anticancer Activity: A Mechanism-Based Review

10

Talambedu Usha, Sushil Kumar Middha, and Kora Rudraiah Sidhalinghamurthy

Contents

Abstract

Cancer is one of the prominent death causing diseases around the globe. About 1 in 6 deaths is due to cancer and its related diseases. Cancer mortality can be reduced by early diagnosis and screening, implementing effective treatments.

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A precise cancer identification is vital for effective treatment, because each cancer type requires a definite treatment procedure, such as radiotherapy, surgery, and chemotherapy. The brisk expansion of herbal therapy and escalating ongoing clinical studies are becoming trendy and useful in the drug development against cancer. Pomegranate (*Punica granatum*) is a prehistoric fruit with illustrious dietary and remedial properties in alternative traditional systems of medicine. The current chapter is aiming to understand various model systems (in silico, in vitro, and in vivo), employed for studying its anti-cancerous properties and diverse molecular effects exhibited by the pomegranate peel and its phytoconstituents. It also highlights the importance of secondary metabolites of *P. granatum*, especially ellagitannins and their anticancer properties. Although there are enormous in vitro and preclinical data, human clinical trials are sorely lacking. The major focus is on up-to-date investigations into the outcomes of previously reported pomegranate peel components against a diverse type of cancers.

Keywords
Cancer · Ellagitannins · Molecular targets · *Punica granatum* · Pomegranate · Treatment

10.1 Introduction

Modern medicine and therapies offer cure for most ailments in today's world, but in some cases, they lead to numerous critical side effects, as seen in the case of cancer therapy (Yin et al. 2013). Nevertheless, much before the use of allopathic medicines, plant-based products were commonly used globally for improving health conditions. There are plenty of functional foods and herbs that are available today, and they possess phytocompounds, which play a vital role in curing a plethora of diseases like jaundice (Kamala et al. 2018), diabetes (Middha et al. 2014; 2019), inflammation (Prashanth Kumar et al. 2019), mouth ulcers, cancer, etc. (Lee et al. 2012). Despite the remarkable advances in diagnostics and therapeutics, cancer (malignancy) is branded to be the deadliest disease. The mortality associated with cancer can be reduced drastically by early diagnosis, intervention, and prevention measures. Epidemiological investigations have indicated that intake of natural products, such as vegetables and fruits, can reasonably decrease the risk of cancer incidence (Donaldson 2004; Syed et al. 2013). There is also a growing use of herbal medicine by cancer patients, due to their safety aspects and cost-effectiveness. Pomegranate (*Punica granatum*), which has been termed as a "Superfood" (Middha et al. 2013a), due to its multiple biological properties is one such plant that holds a great potential for treating cancers (Sharma et al. 2017).

Pomegranate is thought to have taken its root throughout the world, initially being cultivated in Iran, followed by the Himalayan regions of India and different microclimatic zones (Middha et al. 2016). It is a drought-resistant plant that has a long life span approximating up to 200 years with high and healthy fruit yield in the first 20 years (Zarfeshany et al. 2014). The flush, visual appearance, flavor, and

antioxidant competence of the fruit are known to be influenced by the climatic circumstances in which the plant exists. It is a spiny deciduous plant belonging to the plant family, Punicaceae (new classification Lythraceae), which also houses another species *Punica protopunica* (Socotran pomegranate). Punicaceae is placed under the subclass, Rosidae belonging to the order of Myrtales. Pomegranate has small slender leaves, heterostylous funnel-shaped red flowers and the fruit having 5–12 cm diameter with a hexagonal rounded figure amid an upright crown (Singh et al. 2018). The fruiting body can be separated majorly into three parts, namely, rind/husk/pericarp/peel/skin, aril, and the seeds, as shown in Fig. 10.1. It is a plant rich in nutrients and phytochemicals. Be it any part of the pomegranate, i.e., peel, juice, root, bark, flowers, leaves, or seeds, all of them possess potent therapeutic properties. It also targets a range of diseases, including cancer, diabetes, cardiovascular disorders, aging, male infertility, Alzheimer's disease, and Acquired Immune Deficiency Syndrome (AIDS) (Viuda-Martos et al. 2010; Middha et al. 2013a, b). The pomegranate fruit and the other nonedible parts are loaded with anthocyanidins, anthocyanins, flavanols, flavones, flavonones, phenolic acids, and tannins (punicalagin and punicalin). The pharmacological outcome of pomegranate extracts could be linked to their polyphenolic richness (Rummun et al. 2013). The whole genome data have further helped in elucidating genetics, evolution, and other interesting pharmacological effects of pomegranate (Qin et al. 2017).

Pomegranate peel, an affluent natural antioxidant and having varied chemical molecules, was accounted previously for its diverse pharmacological properties such as aging, Alzheimer's disease, cancer, cardiovascular disorders, diabetes, and infertility disorders (Middha et al. 2013a, b; Sun et al. 2016; Singh et al. 2018). This systemic chapter benefits the readers in understanding the usage of pomegranate peel as a natural and alternative medicine in cancer prevention, as it emphasizes the antiproliferative, antimetastatic, and anti-invasive role of pomegranate peel and summarizes its mechanism using an array of cancer cell lines (in vitro), preclinical and clinical models.

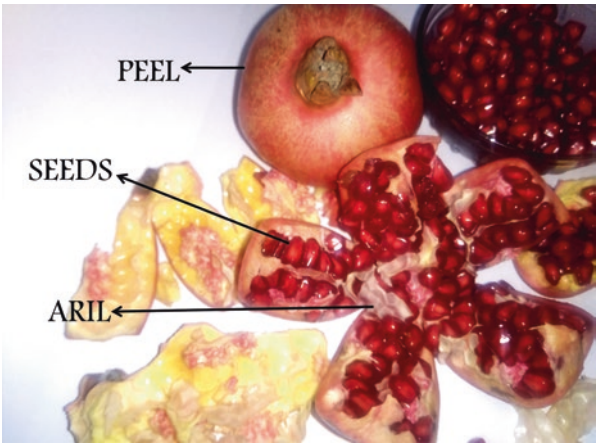

Fig. 10.1 Major parts of pomegranate

10.2 History and Cultural Significance of Pomegranate Peel

Pomegranate is one among the only two species of the kind in *Punica* genus belonging to the family Lythraceae (previously, Punicaceae) (Qin et al. 2017). The common name, *Punica*, is the feminized Latin term for Carthage (the capital town of the prehistoric Carthaginian society, Tunisia). It is originally derived from the Greek Phoinix referring to the Phoenician settlers around Carthage. The precise epithet, granatum, implies seedy or grainy. Prior to its rechristening by Linnaeus in the eighteenth century, the plant was identified as *Malum punicum*, the apple of Carthage (Stover and Mercure 2017). Another representation by few historians stated that the "Tree of Life" in the holy Bible was a pomegranate tree.

The Arabic or Semitic (rumman) and Biblical Hebrew (rimmon) names used for pomegranate mean "fruit of paradise" and has been known to be a symbol of love since ancient times. Pomegranate has been associated with fertility, abundance, immortality, invincibility, blessings, prosperity, posterity, and the endurance of marriage (Stover and Mercure 2017). The Greek physician Soranus documented five prescriptions for the seeds or rind of pomegranate to be used as oral contraceptives or vaginal (douche) suppositories (Foster and Johnson 2006). The contraceptive use of pomegranate seeds or rinds has also been elucidated by Hippocrates (468–377 BCE), Dioscorides (40–90 CE), and Ibn Sina (Avicenna, 980–1037 CE). Historically, pomegranate has been significant in numerous cultures for its food and medicinal uses, as well as for its spiritual and artistic symbolism. The Ebers Papyrus from Egypt (one of the oldest preserved medical documents, ca. 1500 BCE) prescribed it as a remedy for roundworm (Ebbell 1937). Dried fruit rind and pulp have been used commonly for upset stomachs and diarrhea, prepared as infusions (teas) or tinctures (alcoholic extractions) (Van-Wyk and Wink 2004).

Most parts of the pomegranate, including the leaves, fruits, flowers, rind, dried seeds and fresh seeds, trunk bark or root bark, fresh fruits, and preparations thereof (e.g., juice), have defined therapeutic applications in the traditional medicinal systems, such as Ayurveda, Siddha (Traditional Indian), gSo-ba Rig-pa (Traditional Bhutanese), Sowa-Rigpa (Traditional Tibetan), Traditional Chinese Medicine (TCM), Traditional Iranian Medicine, Unani (Perso-Arabic traditional medicine), and European Homeopathy. The fresh fruits and fruit juice are used widely as food, and the juice is also used as a rich source of polyphenols. Essential oils and extracts from different parts of the fruit, as well as isolates and derivatives, such as pomegranate fruit peel extract octenylsuccinate, seed oil hydroxyphenethyl esters, and sterols obtained from the seed oil are used as cosmetic ingredients (Van-Wyk and Wink 2004). In TCM, pomegranate husk/rind/peel (called shi liu pi) is utilized to cure dysentery, diarrhea, rectal prolapse, spermatorrhea, premature ejaculation, uterine bleeding, and vaginal discharge due to kidney instability, and to kill and expel parasites. It is also used topically for ringworm (Bensky 1993) and in combination with other herbs for the conditions mentioned above.

10.3 Characteristics of Pomegranate Peel

The peel is tough and leathery, about 2–5 inches in width, and its color ranges from yellow to deep pink/red. The peel makes almost 50% of the entire mass of the product (fruit) (Fawole et al. 2012). This part of the pomegranate is exceptionally rich in astringent properties and possesses many ethnomedical applications, due to the presence of numerous phytocompounds, but is usually disregarded as agricultural waste (Middha et al. 2013a).

10.3.1 Physicochemical Composition of Pomegranate Peel

The peel of pomegranate is said to be rich in lot of nutrients. The occurrence of total solid, total sugars, reducing sugars, proteins, crude fiber, fat content, and ash is shown to occur in the peels of pomegranate. Apart from these, it is rich in calcium, complex polysaccharides, minerals, nitrogen, magnesium, potassium, phosphorus, and sodium (Middha et al. 2013b).

10.3.2 Phytochemicals of Pomegranate Peel and their Medicinal Properties

Previous studies have shown that the peel is rich in tannins, flavonoids, alkaloids, and organic acids. Tannins, high-molecular-weight polyphenols, are organically and chemically divided into three divergent groups: condensed tannins (also called proanthocyanidins), hydrolyzable tannins or ellagitannins (ETs), and gallotannins (GTs). Pomegranate peel is found to be rich in hydrolyzable tannins, such as punicalin, punicalagin, and other tannins like pedunculagin, gallic acid, and casuarinin are predominantly present. Hence, it possesses superior antioxidative property. Isolariciresinol (10.5 mg/kg) is one of the predominant lignins present in the peel (Syed et al. 2013; Rahmani et al. 2017). Numerous flavonoids, such as catechin, epicatechin, epigallocatechin-3-gallate, flavan-3-ol, kaempferol (Al-Rawahi et al. 2014), kaempferol-3-O-glucoside, kaempferol-3-O-rhamnoglycoside (Akhtar et al. 2015; Moradian et al. 2017), luteolin, luteolin 7-O-glucoside, naringin, pelargonidin, prodelphindin, quercetin (Middha et al. 2013a, b), and rutin, are observed to be present in the peel, as reported from different experimental works (Wang et al. 2004). These phytochemicals attribute to the antibacterial, antioxidant, anti-inflammatory, antiviral, and antineoplastic effects of the peel. Also, there is the existence of gallic acid, gallagyldilacton, and granatin B, which provide anti-inflammatory activity to the peel (Satomi et al. 1993). A plethora of evidence suggests that the presence of these polyphenolics has rendered the peel with the property of anticarcinogenic activity. A total of 108 compounds (Table 10.1) have been reported to be present in the peel through various studies using High Performance Liquid Chromatography (HPLC) and Gas Chromatography–Mass Spectrometry (GC–MS) methods (Syed et al. 2013; Barathikannan et al. 2016; Middha et al. 2013a, b).

Table 10.1 Chemical constituents present in the pomegranate peel

S. no.	Name of the chemical constituent	Molecular formula	References
Polyphenols			
1	Caffeic acid	$C_9H_8O_4$	Viuda-Martos et al. (2010)
2	Valoneic acid dilactone	$C_{21}H_{10}O_{13}$	Jain et al. (2012, b)
3	4,4′-Di-O-methylellagic acid	$C_{16}H_{10}O_8$	Jain et al. (2012, b)
4	3-O-methylellagic acid	$C_{15}H_8O_8$	Jain et al. (2012, b)
5	Brevifolin carboxylic acid	$C_{13}H_8O_8$	Jain et al. (2012, b)
6	*p*-Coumaric acid/4-hydroxycinnamic acid	$C_9H_8O_3$	Viuda-Martos et al. (2010); Mushtaq et al. (2015)
7	Methylgallate	$C_8H_8O_5$	Mushtaq et al. (2015)
8	Caffeoylquinic acid/chlorogenic acid	$C_{16}H_{18}O_9$	Viuda-Martos et al. (2010)
9	Ellagic acid	$C_{14}H_6O_8$	Viuda-Martos et al. (2010); Middha et al. (2013b)
10	Gallic acid	$C_7H_6O_5$	Viuda-Martos et al. (2010); Middha et al. (2013b)
11	Vanillic acid	$C_8H_8O_4$	Mushtaq et al. (2015)
12	Syringic acid	$C_9H_{10}O_5$	Mushtaq et al. (2015)
13	Cinnamic acid	$C_9H_8O_2$	Viuda-Martos et al. (2010)
14	Ferulic acid	$C_{10}H_{10}O_4$	Mushtaq et al. (2015)
15	Sinapic acid	$C_{11}H_{12}O_5$	Mushtaq et al. (2015)
16	p-Coumaric acid glucuronide	–	Mushtaq et al. (2015)
17	Hydroxybenzoic acid	$C_7H_6O_3$	Viuda-Martos et al. (2010)
Tannins			
1	Punicalin	$C_{34}H_{22}O_{22}$	Viuda-Martos et al. (2010); Fischer et al. (2011)

(continued)

Table 10.1 (continued)

S. no.	Name of the chemical constituent	Molecular formula	References
2	Punicalagin	$C_{48}H_{28}O_{30}$	Zahin et al. (2010); Middha et al. (2013b); Y-q et al (2017)
3	Granatin A	$C_{34}H_{24}O_{22}$	Seeram et al. (2005)
4	Granatin B	$C_{41}H_{28}O_{27}$	Seeram et al. (2005)
5	Pedunculagin	$C_{34}H_{24}O_{22}$	Seeram et al. (2005)
6	Punicalin	$C_{34}H_{22}O_{22}$	Seeram et al. (2005)
7	Corilagin	$C_{27}H_{22}O_{18}$	Satomi et al. (1993)
8	Castalagin	$C_{41}H_{26}O_{26}$	Satomi et al. (1993)
9	2.3-(S)-Hexahydroxydiphenoyl (HHDP)-d-glucose	$C_{20}H_{18}O_{14}$	Satomi et al. (1993)
10	Gallagic acid	$C_{28}H_{14}O_{18}$	Satomi et al. (1993)
11	Casuarinin	$C_{41}H_{28}O_{26}$	Satomi et al. (1993)
12	*Caffeoylquinic acid or Chlorogenic acid*	$C_{16}H_{18}O_9$	Arnoni et al. (2015)
13	Tellimagrandin	$C_{41}H_{30}O_{26}$	Barathikannan et al. (2016)
14	Prodelphindin	$C_{30}H_{26}O_{13}$	Barathikannan et al. (2016)
Gallotannins			
1	*Digalloyl-hexoside/Glucogallin*	$C_{13}H_{16}O_{10}$	Fisher et al. (2011))
2	*Monogalloyl-hexoside/Glucogallin*	$C_{13}H_{16}O_{10}$	Fisher et al. (2011)
Flavonoids			
1	Cyanidin (anthocyanins)	$C_{15}H_{11}O_6^+$	Viuda-Martos et al. (2010)
2	Delphinidin (anthocyanins)	$C_{15}H_{11}ClO_7$	Viuda-Martos et al. (2010)
3	Pelargonidin-3-O-glucoside (anthocyanins)	$C_{21}H_{21}ClO_{10}$	Viuda-Martos et al. (2010)
4	Catechin	$C_{15}H_{14}O_6$	Wafa et al. (2017)
5	Gallocatechin	$C_{15}H_{14}O_7$	Wafa et al. (2017)
6	Procyanidin B	$C_{30}H_{26}O_{12}$	Wafa et al. (2017)

(continued)

Table 10.1 (continued)

S. no.	Name of the chemical constituent	Molecular formula	References
7	Myricetin	$C_{15}H_{10}O_8$	Wu and Tian. (2017)
8	Quercetin	$C_{15}H_{10}O_7$	Middha et al. (2013b)
9	Kaempferol (Flavonols)	$C_{15}H_{10}O_6$	Wafa et al. (2017)
10	Luteolin (flavone)	$C_{15}H_{10}O_6$	Wafa et al. (2017)
11	Apigenin (flavone)	$C_{15}H_{10}O_5$	Wu and Tian. (2017)
12	Rutin (O-glycosides)	$C_{27}H_{30}O_{16}$	Middha et al. (2013b)
14	Cyanidin-3-rutinoside (anthocyanins)	$C_{27}H_{31}O_{15}^+$	Wafa et al. (2017)
15	Cyanidin-3-pentoside (anthocyanins)		Wafa et al. (2017)
16	Cyanidin-3,5-diglucoside (anthocyanins)	$C_{27}H_{31}O_{16}^+$	Wafa et al. (2017)
17	Cyanidin-3-O-glucoside (anthocyanins)	$C_{21}H_{21}O_{11}^+$	Wafa et al. (2017)
18	Cyanidin 3-rutinoside (anthocyanins)	$C_{27}H_{31}O_{15}^+$	Wafa et al. (2017)
19	Delphinidin 3,5-diglucoside (anthocyanins)	$C_{27}H_{31}O_{17}^+$	Wafa et al. (2017)
20	Delphinidin 3-glucoside	$C_{21}H_{21}O_{12}^+$	Wafa et al. (2017)
21	Pelargonidin	$C_{15}H_{11}O_5^+$	Middha et al. (2013b)
22	Pelargonidin-3,5-diglucoside (anthocyanins)	$C_{27}H_{31}ClO_{15}$	Fisher et al. (2011)
23	Pelargonidin-3-glucoside	$C_{21}H_{21}O_{10}^+$	Fisher et al. (2011)
24	Delphinidin 3-glucoside (anthocyanins)	$C_{21}H_{21}O_{12}^+$	Fisher et al. (2011)
Alkaloid			
1	Pelletierine	$C_8H_{15}NO$	Barathikannan et al. (2016)
Tocopherols			
1	α-Tocopherol, γ-tocopherol, and δ-tocopherol/vitamin E	$C_{29}H_{50}O_2$	Elfalleh et al. (2011)
Terpene			
1	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]	$C_{15}H_{24}$	Barathikannan et al. (2016)
Unclassified category			
1	Heptadecane	$C_{17}H_{36}$	Barathikannan et al. (2016)

(continued)

Table 10.1 (continued)

S. no.	Name of the chemical constituent	Molecular formula	References
2	Triacotane	$C_{31}H_{64}$	Barathikannan et al. (2016)
3	Octadecane	$C_{18}H_{38}$	Barathikannan et al. (2016)
4	Squalene	$C_{30}H_{50}$	Barathikannan et al. (2016)
5	Eicosane	$C_{20}H_{42}$	Barathikannan et al. (2016)
6	5-Hydroxymethylfurfural (furan)	$C_6H_6O_3$	Barathikannan et al. (2016)
7	4-Mercaptophenol/4-Hydroxythiophenol	C_6H_6OS	Barathikannan et al. (2016)
8	Quinic acid	$C_7H_{12}O_6$	Barathikannan et al. (2016)
9	Heneicosane	$C_{21}H_{44}$	Barathikannan et al. (2016)
10	5-Hydroxymethylfurfural	–	Barathikannan et al. (2016)
11	4-Fluorobenzyl alcohol	–	Barathikannan et al. (2016)
12	Hexadecane, 1-iodo-Hexadecane Nonane	–	Barathikannan et al. (2016)
13	Z-8-Hexadecane, 9-Eicosene, (E)-n-Pentadecanol	–	Barathikannan et al. (2016)
14	Alpha-Copaene, alpha-Cubebene	–	Barathikannan et al. (2016)
15	Hexadecane, 2-Bromotetradecane	–	Barathikannan et al. (2016)
16	Heneicosane, 11-pentyl-Docosane, 11-butyl-Tridecane	–	Barathikannan et al. (2016)
17	Nonadecane, 9-methyl-Nonane, 5-butyl-Heptadecane	–	Barathikannan et al. (2016)
18	Z-8-hexadecane, Pentafluoropropionic acid, 4-hexadecyl ester	–	Barathikannan et al. (2016)
19	Nonadecane, 9-methyl, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione.	–	Barathikannan et al. (2016)
20	Pentadecanoic acid, 14-methyl aster, Hexadecanoic acid, methyl ester	–	Barathikannan et al. (2016)
21	Nonadecane, 9-methyl, Eicosane, Pentacosane	–	Barathikannan et al. (2016)
22	1-Heneicosyl formate, Cyclooctacosane, (Z)-9-Tricosen	–	Barathikannan et al. (2016)
23	Triacontane, 1-bromo-1-Chloroeicosane Heptadecane	–	Barathikannan et al. (2016)
24	Dodecane, 2,6,11-trimethyl-docosane, 7-hexyl-Tetracosane	–	Barathikannan et al. (2016)

(continued)

Table 10.1 (continued)

S. no.	Name of the chemical constituent	Molecular formula	References
25	Linoleic acid ethyl ester, n-Propyl 9,12-octadecadienoate, 9,12-octadecadienoic acid, ethyl ester	–	Barathikannan et al. (2016)
26	1-Nonadecene, 9-Trocosene, (Z)-Bacchotricuneatin	–	Barathikannan et al. (2016)
27	1-Nonadecene, 9-Trocosene, Z-5-Nonadecene	–	Barathikannan et al. (2016)
28	Heptadecane	–	Barathikannan et al. (2016)
29	6-Octen-1-ol, 3,7-dimethyl acetate Phytol, acetate 1,2–15, 16-Diepoxyhexadecane	–	Barathikannan et al. (2016)
30	3,5,7-Tricyclopropyl-5,6-dihydro-5-methyl-1,2 (4H)-diazepineOctanoic acid, but-3-yn-2-yl ester Ethisterone	–	Barathikannan et al. (2016)
31	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-Bicyclo [10.1.0] trideca-4,8-diene-1 3-carboxamide,N-(3-chlorophenyl)-1H-2, 8a-Methanocyclopenta[a]cyclopropa[e] cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxyl-1,4-bis(hydroxymethyl)-1,7,9-trimethyl [1S-(1.Alpha., 1a.Alpha., 2.Alpha., 5. Beta.,5a.Beta., 6.Beta., 8a.Aipha., 9. Alpha., 10a.Alpha.)]	–	Barathikannan et al. (2016)
32	Heptadecane, 3-methyl-Octadecane, Nonadecane	–	Barathikannan et al. (2016)
33	Octacosane, Tetracosane	–	Barathikannan et al. (2016)
34	Hexatriacontane, Octadecane, 1-iodo-Tetracontane	–	Barathikannan et al. (2016)
35	1-Hexacosene, 9-hexacosene, E-15-heptadecenal	–	Barathikannan et al. (2016)
36	CyclobarbitalTris(tert-butyldimethylsilyloxy)arsane, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro isopropyl ester	–	Barathikannan et al. (2016)
37	2,4-Cyclohexadien-1-one, 3,5-bis, 1-dimethylethyl-4-hydroxy-Tetrasiloxane, decamethyl-Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl	–	Barathikannan et al. (2016)
38	Anthracene, 9,10-dihydro-9,9,10-trimethyl-1H-Indole, 1-methyl-2-phenyl-Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyralyl)	–	Barathikannan et al. (2016)

(continued)

Table 10.1 (continued)

S. no.	Name of the chemical constituent	Molecular formula	References
39	N-Methyl-1-adamantaneacetamide Arsenous acid, tris(trimethylsilyl)ester, Benzo[h]quinolone, 2,4-dimethyl	–	Barathikannan et al. (2016)
40	9,19-Cyclolanost-24-en-3-ol, Lanosterol, Lanost-7-en-3-one	–	Barathikannan et al. (2016)
42	Tirucallol, Lanosterol, D:B-Friedo-18, 19-secolup-19-ene, 10-epoxy	–	Barathikannan et al. (2016)
43	1,2-Bis(trimethylsilyl) benzene, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramineBenzo[h] quinolone, 2,4-dimethyl	–	Barathikannan et al. (2016)
44	1H-Indole, 1-methyl-2-phenyl-Arsenous acid, tris(trimethylsilyl) ester, Cyclotrisiloxane, hexamethyl	–	Barathikannan et al. (2016)
45	Furan-2-carboxamide, N-(3-nitrophenyl)-1-propanone, 1-(2-furanyl)-4-pyrridinol	–	Barathikannan et al. (2016)
46	Benzene, 1,3-bis(1,1-dimethylethyl), benzene, 1,4-bis(1,1-dimethylethyl)	–	Barathikannan et al. (2016)
47	5-Hydroxymethylfurfural, 4-fluorobenzyl alcohol	–	Barathikannan et al. (2016)
48	5-methyl-2-phenylindozine (1H) Pyrrole-3-carboxylic acid, 5-[cyano(4-morpholinyl) methyl]-1-(methoxymethyl), methyl ester 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	–	Barathikannan et al. (2016)

10.4 Anticancer Activity of Pomegranate Peel

The World Health Organization (WHO) and the American Cancer Society (ACS) reported that globally the second growing fatal disease is cancer, claiming approximately 9.6 million mortality in 2018. Around 1 in 6 demises worldwide is caused by malignancy and roughly 70% of these mortalities transpire in low- and middle-revenue nations. According to the ACS 2018 statistics in the United States (USA), an approximated 1,735,350 latest cancer cases in various hospitals/institutions and 609,640 cancer demises are witnessed (http://www.who.int/news-room/ fact-sheets/detail/cancer).

The currently available cancer treatments include radiotherapy, surgery, and systemic cures comprising cytotoxic chemotherapy, hormonal remedy, immunotherapy, and intentioned or targeted therapies such as hydrogels and magnetogels (Veloso et al. 2018). Although numerous therapies are available, all of them have their own side effects and some of them even create a financial burden on the patient. So, there is an urgent need for definitive preventive measures and complete cure for cancer, which will have lesser systemic toxicity as well as one that will not drill a

hole in the patient's pocket (Thomford et al. 2018). Recently, the advances in novel cancer remedies have become a major issue owing to the cells developing resistance to modern chemotherapy. Alternatively, in the present time, an herbal therapy is in focus owing to its less costs and toxicity. The current chapter attempts to summarize the literature available in PubMed and Google Scholar on the various model systems (in silico, in vitro, and in vivo) utilized to examine the anticancerous activities of peel and different molecular effects exhibited by the pomegranate peel (Table 10.2). A number of studies have centered on the anticancerous properties of pomegranate peel using in silico, in vitro, and in vivo model systems. The early scientific evidences majorly focused on the antidiarrheal activity of pomegranate peel and its genotoxicity studies. For the first time, Settheetham and Ishida (1995) reported that the administration of pomegranate aqueous extract encouraged DNA fragmentation during apoptosis in Raji and P3HR-1 cells. Though there was a huge gap until 2004, the later years have witnessed a drastic increase and interest in the anticancer and other pharmacological properties of pomegranate peel. This chapter also focuses on the demand for pomegranate peel and its derived compounds and their anticancer properties. Interestingly, the extracts of pomegranate peel have shown a selective inhibition against various types of cancer cells with no or less visible toxicity in normal cells.

10.4.1 Pomegranate Peel Against Breast Cancer

Breast cancer is a very commonly occurring cancer type in females around the world with an impact on more than 1.5 million women every year. It is also a reason for the massive number of cancer-related fatalities in women. A plethora of scientific evidence suggests that the consumption of phytochemicals-rich food can trim down the threat of cancer disease. Pomegranate peel extract (PPE), the biowaste material, is rich in polyphenols. Ricci et al. (2006) reported that the fruit juice has a higher polyphenolic content (0.063–0.0003 mg/g dry weight) than different varieties of the pomegranate peel (1.892–0.1070 mg/g dry weight) (Dikmen et al. 2011). In vitro and in vivo explorations have divulged that PPE, rich in polyphenols, act as potent antioxidants, which help in the inhibition of cell growth in cancer. Jeune et al. (2005) performed a study that demonstrated the in vitro anticancer effects of combining pomegranate extracts and genistein. They also proposed that the combination of both is more efficacious, as compared to single treatments in a time- and dose-dependent manner. They used lactate dehydrogenase, 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium bioassays, acridine orange–ethidium bromide, and terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labeling to study the cytotoxic and growth inhibition effects of pomegranate extracts and genistein on MCF-7 cancer cells. Similar studies were also carried out by Dikmen et al. (2011) to demonstrate that the methanolic extract of pomegranate peel at varied concentrations (25, 50, 100, 200, and 300 μg/ml)

Table 10.2 Summary of molecular targets and anticancer potential of the pomegranate peel

Type of cancer	Model used (cells/animals)	Target pathway	References
Breast cancer	MDA-MB-231 cells	Decreases the gene expression of vimentin gene, zinc finger E-box-binding homeobox 1 (ZEB1), and β-catenin Increases the expression of E-cadherin Inhibits epithelial mesenchymal transition and metastasis	Bagheri et al. (2018)
	MCF-7, PC-3, and HepG-2	Solid lipid nanoparticles of pomegranate peel reduce the cell growth	Badawi et al. (2018)
	MCF-7 cancer cell line	Monodisperse platinum nanoparticles (Pt NPs) biosynthesized from pomegranate peel inhibit cell proliferation with a IC50 of 17.84 μg/ml after 48 h of incubation	Sahin et al. (2018)
	MCF-7 cell line	Silver nanoparticles of pomegranate peel inhibit the proliferation of cell at a dose of 12.85 μg/ml	Sahin et al. (2017)
	MCF-7 cell line	Downregulates the estrogen response element (ERE)-mediated transcription in breast cancer cells	Vini et al. (2016)
	MCF-7 cell line	Exhibits antiproliferative activity	Modaeinama et al. (2015)
	MCF-7 cell line	Increases the expression of Bax (pro-apoptotic gene) and reduces B-cell lymphoma 2 (Bcl-2) expression (anti-apoptotic gene) cell proliferation and induces apoptosis on MCF-7 cancer cells	Dikmen et al. (2011)
	Human breast MCF-7	Methanolic and acetone extract of pomegranate peel exhibits antiproliferative activity	Fazio et al. (2018)
	Human metastatic breast cancer cell line – MDA-MB-231	Upregulates the expression of intercellular adhesion molecule 1 (ICAM-1) Downregulates the expression of matrix metallopeptidase-9 (MMP-9), fibronectin, and vascular endothelial growth factor (VEGF)	Ahmadiankia et al. (2018)

(continued)

Table 10.2 (continued)

Type of cancer	Model used (cells/animals)	Target pathway	References
Colorectal cancer	Female dark Agouti rats (100–140 g, 6 weeks old)	Ameliorates 5-FU-induced intestinal mucositis	Chen et al. (2018)
	HT-29 CRC cell line	Induces intrinsic apoptosis with a decrease in mitochondrial potential, increases bcl-2-like protein 4 (BAX) to Bcl-2 ratio, and cleaves caspase-9 and caspase-3	
	Apc-mutated Pirc rats	Exhibits pro-apoptotic and anti-inflammatory action	Tortora et al. (2018)
	HT29 cells	Reduces cyclooxygenase-2 (COX-2) protein expression by 70% shows increase in caspase-3 (CASP-3) expression in cells	
Colon cancer	Colon (LoVo) cancer cell lines	Reduces the cell proliferation	Moreira et al. (2017)
	RKO: ATCC® CRL-2577™ cells	12.5 µg of silver nanoparticles from *Punica granatum* peel caused reduction in cell proliferation with viabilities of 56% and 61% on days 3 and 5, respectively.	Devanesan et al. (2018)
Prostate cancer	TRAMP-C1 DU145 and PC3 cells	Reduces mitochondrial transmembrane potential (Δym) Helps in the accumulation of reactive oxygen species (ROS) Induces apoptosis Impairs metastasis by downregulating matrix metalloproteinase-2/matrix metalloproteinase-9 (MMP-2/MMP-9) and upregulating tissue inhibitor of Metallopeptidase inhibitor 2 (TIMP2)	Deng et al. (2017)
	PC-3 cells	Exhibits antiproliferative activity	Modaeinama et al. (2015)
	LNCaP-AR and LAPC4 cells	Mediates nuclear factor κappa-light-chain-enhancer of activated B cells (NF-κB) blockade Reduction in S-phase fraction and accumulation of cells in the G1 phase	Retti et al. (2008)
	SCID mice implanted with LAPC4 prostate cancer LAPC4 xenograft model	Inhibits the growth of androgen-independent LAPC4 xenografts with an increase in Ki63 (proliferation marker) Enhances apoptosis Reduces phospho-IκBα levels	

(continued)

Table 10.2 (continued)

Type of cancer	Model used (cells/animals)	Target pathway	References
Cervical cancer	HeLa cells	Ellagic acid from peel inhibition of cervical cancer by promoting IGFBP7 expression Inhibits the protein kinase B/mammalian target of rapamycin (AKT/mTOR) signaling pathway by enhancing the expression level of insulin-like growth factor binding protein 7 (IGFBP7)	Guo et al. (2016)
Hepatocellular carcinoma	Male albino rats exposed to the hepatocarcinogen diethylnitrosamine (DENA)	Decreases the tumor size, liver index, and the anti-apoptotic protein Bcl-2 in liver Increases the amount of glutathione in liver Exhibits antimutagenic effect	El-Ashmawy et al. (2016)
	Hep-G2 cells	Cell cycle of HepG-2 arrested at the S-phase by inducing mitochondrial apoptotic pathway in a dose-dependent manner Increases Cyt-c 32 and the activity of Caspase-3/9 ROS levels increased Increases the ratio of Bax/Bcl-2 Increases protein expressions of P53	Song et al. (2016)
Urinary bladder cancer	Bladder cancer T24 cells	Ethyl acetate extract of pomegranate peel exhibits antiproliferative activity	Masci et al. (2016)
	EJ bladder cancer cells	Suppresses the EJ cell proliferation Promotes caspase-dependent apoptosis Decreases the expression of c-Jun and increases the expression of p53 The c-Myc and CD44 are the direct targets of MicroRNA-34a (miR-34a) in EJ cell apoptosis induced by the peel.	Zhou et al. (2015)
	Balb C nude mice	Decreases the tumor growth volume with no toxicity in the liver, spleen, and intestine of mice	
Ovarian cancer	SKOV3 cells	Inhibits increase in the uterine weight	Sreeja et al. (2012)
	Ovariectomized Swiss albino mice	Decreases cell proliferation in ovariectomized mice	
	SK-OV-3 cells	Exhibits antiproliferative activity	Deng et al. (2017)
Chronic myeloid leukemia	K562 cells	Promotes the growth inhibition of K562 cells, mainly via the G2/M phase arrest Upregulates caspases and cytochrome c Elevates the expression of p21 and p53	Asmaa et al. (2015)

(continued)

Table 10.2 (continued)

Type of cancer	Model used (cells/animals)	Target pathway	References
Lung cancer	A549 (lung nonsmall cell cancer)	Exhibits antiproliferative activity	Modaeinama et al. (2015)
Osteosarcoma	U-2 osteosarcoma (OS) cells	Induces the arrest of G2/M phase Induces apoptosis through the intrinsic mitochondrial pathway Inhibits the growth of cells in a dose-dependent manner Shows increase in the Bax/Bcl-2 ratio Decreases the mitochondrial membrane potential, release of cytochrome c, activation of caspase-9 and caspase-3, and cleavage of poly-(ADP-ribose)-polymerase (PARP)	Li et al. (2014)
Thyroid cancer	Human papillary thyroid cancer cell lines BCPAP (harboring BRAF V600E mutation) TPC-1 cell lines Nthy-ori 3–1 (human thyroid follicular epithelial cell line)	Inhibits proliferation and influences the morphology of thyroid cancer cells Induces cell apoptosis Induces the loss of mitochondrial transmembrane potential ($\Delta\Psi m$) and ROS generation Decreases the expression of MMP-9 and indicates the inhibition of thyroid cancer cell migration and invasion	Li et al. (2016)
	BALB/c Nude mice (5–6 weeks old)	Tumor volumes and weight significantly decreased Increases cleaved caspase-3 (CC-3)-positive cells And decreases Ki-67-positive cells No pathologic changes were observed in the heart, liver, spleen, lung, and kidney after the pomegranate peel treatment	
Skin cancer	Human epidermal keratinocytes and dermal fibroblasts	Stimulates type I procollagen synthesis and inhibits matrix metalloproteinase-1 (MMP-1; interstitial collagenase) production by dermal fibroblasts that promote the regeneration of dermis	(Aslam et al. 2006)

(continued)

Table 10.2 (continued)

Type of cancer	Model used (cells/animals)	Target pathway	References
Lung cancer, gastric cancer, prostate cancer, breast cancer, and liver cancer	In silico study	Quercetin, one of the chemical constituents, inhibits the following proteins/receptors: GTPase HRas, proto-oncogene tyrosine-kinase Src, tyrosine-protein kinase HCK, HSP 90-alpha, cell division protein kinase 2, basic fibroblast growth factor receptor 1, Cyclin A2, glycogen synthase kinase 3 beta, estradiol 17-beta-dehydrogenase 1, leukotriene A-4 hydrolase, lysozyme C, death-associated protein kinase 1, vitamin D3 receptor, apoptosis regulator BCL-X, proto-oncogene lymphocyte-specific protein tyrosine-protein kinase (LCK), serine/threonine-protein kinase polo-like kinase 1 gene (PLK1), Serine/threonine (Ser/thr) protein kinase, cell division protein kinase 9, casein kinase II subunit alpha, Cyclin-dependent kinase 6, proto-oncogene tyrosine-protein kinase receptor RET, androgen receptor, NAD(P)H dehydrogenase [quinone]	(Usha et al. 2015)

Note: *MDA-MB* M.D. Anderson-metastasis breast cancer, *MCF-7* Michigan cancer foundation-7, *PC-3* prostate cancer 3, *HepG-2* liver hepatocellular cells, *IC50* half-maximal inhibitory concentration, *HT-29* CRC human colorectal adenocarcinoma cell line, *Apc-Mutated* adenomatous polyposis coli, *PC3* human prostate cancer cell line, *LAPC4* los angeles prostate cancer-4, *SCID* severe combined immunodeficiency, *LNCaP* metastatic lesion of human prostatic adenocarcinoma

decreased cell proliferation and stimulated apoptosis in MCF-7 cancer cells. This was evident from the enhanced expression of pre-apoptotic gene Bax and decrease in the expression of anti-apoptotic gene Bcl-2. The effect of PPE was proportional to the dose and the incubation interval. Bcl-2/Bax plays a vital function in regulating caspase-dependent and caspase-independent apoptosis mediated by the mitochondrial pathway. The possible anticancer and apoptotic effects of pomegranate peel can be credited to ellagic acid, ellagic tannin, and gallic acid. The data gathered from both these studies aid to invent new chemotherapeutic and chemopreventive agents of pomegranate peel to treat breast cancer.

10.4.2 Pomegranate Peel Against Colorectal Cancer

Colorectal cancer (CRC) is the anomalous division of cells that occur in the colon or rectum or colorectum, also called the large intestine. As per the Cancer Statistics 2018 report, colorectal cancer is the third most familiar cancer to be detected equally

in both men and women in the United States. The WHO reports approximately 862,000 CRC deaths across the globe in 2018 (https://www.who.int/news-room/fact-sheets/detail/cancer). Siegel et al. (2016) reported that roughly 4.6% of man (1 in 22) and 4.2% of women (1 in 24) will be identified with CRC in their life span. Waly et al. (2012) observed an improvement in the redox status and decrease in preneoplastic lesions of the colonic cells in azoxymethane (AOM)-induced in vivo colon tumors treated with PPE. The proposition that PPE extracts might prevent colon cancer was based on in vitro studies that showed the high antioxidant properties of pomegranate peel. Increasing evidences have implicated that the augment in reactive oxygen species (ROS) is one of the causes of cell damage and cancer. In this context, Negi et al. (2003) used two strains of *Salmonella typhimurium* (*S. typhimurium)*, i.e., TA100 and TA1535, to test the efficacy of PPE extracts against sodium azide mutagenicity. The study revealed that strong antimutagenicity of soxhlet extracts of water, methanol, and ethyl acetate pomegranate peel at 2500 μg decreased mutagenicity in both strains of *Salmonella* species. The antioxidant and antimutagenic effects could be the result of the polyphenols, such as catechins, chlorogenic, caffeic, ellagic acid, and ferulic acids, present in the peel.

10.4.3 Pomegranate Peel Against Prostate Cancer

Prostate cancer (Pca) is accountable for the highest number of cancer cases reported in men. It accounts for every 1 in 5 new cancers diagnosed in men according to the American Cancer Society (Siegel et al. 2016). According to Cancer Statistics 2018 reports of the American Institute for Cancer Research, prostate cancer is the second most lethal cancer spotted in men with 1.3 million new cases in the United States (https://www.wcrf.org/dietandcancer/cancer-trends/prostate-cancer-statistics). Deng et al. (2017) examined the in vitro outcomes of *Punica* peel on Pca cells and further provided evidence for its application to inhibit the Pca growth and metastasis. The study was inspired by Venclexta, a drug used to treat chronic lymphocytic leukemia (CLL), approved by the US FDA on April 11, 2016. Venclexta is a Bcl-2 inhibitor that specifically targets the apoptosis pathway in prostate cancer cell lines, DU145, PC3, and the mouse prostate cancer cell TRAMP-C1 (Ng and Davids 2014; Deng et al. 2017). HPLC analysis showed the presence of two compounds, namely punicalagin (PG) and ellagic acid (EA), with a molecular weight of 1083.0 and 301.0, respectively, as determined by mass spectrophotometry. The viability of cells was evaluated by a routine 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) test. The morphological analysis of nuclei by Hoechst staining showed the inhibition of cell viability by the *Punica* peel extract attributed to apoptosis. The content of punicalagin (479.8 mg/g) and ellagic acid (7.5 mg/g) in *Punica* was recorded based on the regression equation and the relevant area under the curve (AUC) of each factor. The antiproliferative effect was significantly seen in TRAMP-C1, compared to DU145 and PC3 cells. The results also showed a reduced expression of anti-apoptotic Bcl-2 and amplified expression of cleaved caspase-3 and pro-apoptotic Bax posttreatment, indicating mitochondrial dysfunction causing

apoptosis. *Punica* peel treatment also showed decreased mitochondrial transmembrane potential and ROS production. The results also indicated that after treatment with peel extract, the expression levels of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) in TRAMP-C1 were significantly suppressed and tissue inhibitor of metalloproteinases-2 (TIMP2) was upregulated indicating the inhibition and invasion, the two important steps in cancer metastasis. Therefore, the study clearly demonstrates that the *Punica* peel presents a clear inhibitory effect on the growth and viability of prostate cancer cell lines.

A subcutaneous xenograft of human prostate cancer cells (PC-3) in nude mouse models was established by Ma et al. (2015) to observe the antiproliferative and apoptotic effects of pomegranate peel polyphenols. Pomegranate peel helped in shrinking tumor dimensions and mass in tumor-bearing nude mice, and significantly enhanced the rate of apoptosis. In addition, tumor necrosis factor (TNF)-α was amplified and vascular endothelial growth factor (VEGF) in serum was decreased. The study showed the antitumor activity of three polyphenols ellagic acid, gallic acid, and punicalagin found in pomegranate peels.

A plethora of literature has described the association between inflammation and prostate carcinogenesis (Kohnen and Drach 1979). The nuclear factor-κB (NF-κB) pathway is one of the well-established signaling pathways that arbitrate cancer-related inflammatory responses (Baldwin Jr 2001). Contagious NF-κB activation has been learnt in breast, cervical, liver, melanoma, and prostate cancers. Importantly, constitutive activation of NF-κB in primary prostate cancer specimens represents an independent risk factor for tumor recurrence after surgery. Rettig et al. (2008) indicated the suppression of NF-κB signaling followed by *Punica granatum* peel extract treatment in both in vitro and in vivo prostate cancer models (Rettig et al. 2008). In vitro stimulation of apoptosis by peel was demonstrated and found to be dependent on the inhibition of NF-κB activity. For in vivo studies, SCID mice implanted with LAPC4 prostate cancer peel impedimented (delayed) the emergence of LAPC4 androgen-independent xenografts in castrated mice through cell proliferation inhibition and apoptosis induction.

10.4.4 Pomegranate Peel Against Skin Cancer

Skin cancer prevalence has increased among light-skinned populations and artificial tanning devices have significantly contributed to this increase over the last three decades (http://www.who.int/bulletin/volumes/95/12/17-021217/en/. Accessed 12 Dec 2018). One in five Americans is prone to skin cancer in their life span according to the American Skin Cancer Foundation Statistics (http://www.who.int/uv/faq/skincancer/en/index1.html. Accessed 12 Dec 2018). Increasing incidence of skin cancer provide a strong basis for chemoprevention with natural remedies. Aslam et al. (2006) first reported the ability of the aqueous fraction of pomegranate peel to advance propagation and procollagen synthesis and MMP-1 inhibition in organ cultures developed from punch biopsies of sun-confined hip skin attained from adults.

10.4.5 Pomegranate Peel Against Thyroid Cancer

Thyroid cancer is a cancer of the thyroid glands and is of four different types—papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), medullary thyroid cancer (MTC), and anaplastic thyroid cancer (ATC). The thyroid cancer survival rate, especially PTC, is relatively higher than any other malignancy. Thyroid cancers are majorly treated with surgical procedures that profusely affect the patient's quality of life and it also requires patients to adhere to stern indications and contraindications. All these abovementioned conditions call for latest clinical interventions in thyroid cancer treatment. The earliest evidence for the utilization of pomegranate peel in the prevention and treatment of thyroid cancer came from studies carried out by Li et al. (2016). They evaluated the effect of pomegranate peel on thyroid cancer cells, such as BCPAP (harboring BRAF V600E mutation), TPC-1 (harboring RET-PTC rearrangement), and Nthy-ori 3–1 (human thyroid follicular epithelial cell line). The outcome of the study showed a significant decrease in the propagation of thyroid cancer cells in a time- and dose-dependent manner.

The preliminary mechanism was identified to be the induction of intrinsic apoptosis by Bcl-2 and Bax proteins and a decrease in mitochondrial membrane potential by PG and EA. Similar studies were conducted by the same group in vivo in the BCPAP tumor model in BALB/c athymic mice. *Punica* peel (dosage of 125 mg/kg/d) showed antitumor activity with 69.8% inhibition of tumor growth, improved the expression of cleaved caspase-3 (CC-3), and suppressed the expression of Ki-67 (a protein) in cancerous tissues in comparison with the untreated groups. Thyroid cancer can also metastasize to the lymph nodes and lungs. Restraining the tumor cell from migrating and invading other organs is a useful approach to inhibit metastasis, and *Punica* peel significantly downregulates the expression of MMP-9, which is one of the important proteins in the focal adhesion kinase (FAK)/MMP pathway, a vital pathway in tumor assault/invasion and metastasis.

10.4.6 Pomegranate Peel Against Osteosarcoma

Osteosarcoma is a high-grade primary skeletal sarcoma and is characterized by the deposition of immature osteoid matrix by mesenchymal spindle cells. In a study by Li et al. (2014), a homogeneous acidic polysaccharide was isolated from the pomegranate peel and its antiproliferative activity against human U-2 osteosarcoma (U-2OS) cells examined. The same study also elucidated the chemical composition of a pomegranate peel. It was shown to contain total sugar (72.4%), uronic acid (19.5%), and a negligible quantity of protein (9.7%). Further, the pomegranate peel was effective in inducing the arrest of G2/M phase, encouraged apoptosis, and hindered the growth of U-2OS cells in a dose-dependent manner. Western blotting analysis displayed that the pomegranate peel elicited the mitochondrial-mediated apoptosis, which was evident from the elevated levels of Bax/Bcl-2 ratio, release of cytochrome c, triggering of caspase-3 and caspase-9, and cleavage of poly-(ADP-ribose)-polymerase (PARP) in U-2OS cells.

10.5 Punicalagin and Ellagic Acid Isolated from Pomegranate Peel with Anticancer Potential

Punicalagin (PG) and ellagic acid (EA) are the two major constituents of pomegranate peel. Studies by Zahin et al. (2014) have clearly demonstrated in vitro antimutagenic and antiproliferative effects of these compounds in human lung cancer cells. Ellagitannins are the abundant polyphenols present in the peels of pomegranate. Punicalagin, a unique ellagitannin of pomegranate, has the capability to be hydrolyzed to ellagic acid ending in prolonged release of ellagic acid into the blood after the ingestion of pomegranate peel. It is known to be the largest polyphenol, having a molecular weight greater than 1000 g/mol. Punicalagin is most abundant in the peel/rind, compared to seeds/fruit (Lu et al. 2007). Structurally similar to gallagic acid and ellagic acid moiety, punicalagins are attached to glucose and exist in two reversible anomer types, i.e., α and β forms. Punicalagin can inhibit sulfoconjugation (Saruwatari et al. 2008) and has antioxidant (Sun et al. 2016), antiproliferative, antigenotoxic, antiviral, antiplasmodial, and immunosuppressive activity (Aqil et al. 2012; Sharma et al. 2017). Ellagitannins are also metabolized into bioactive urolithins by gastrointestinal (gut) microbiota, which conjugate in the hepatic lobes and are urinated out. These urolithins are known to reduce prostate cancer intensification (Heber 2008).

Ellagic acid (EA) is a polyphenolic molecule present in a variety of vegetables and fruits, and pomegranate peel is one among them. It is usually believed as an antioxidant. Clinical tests with EA on cultured cell lines (Human) revealed the prevention of denaturation of the p53 gene. Further, another study proposed that one of the mechanisms by which EA hinders mutagenesis and carcinogenesis is by the development of adducts with DNA, camouflaging binding sites from the mutagen or carcinogen (Sharma et al. 2017). The advent of next-generation sequencing techniques has improvised the overall understanding of biosynthesis of secondary metabolites in different plant species. The whole genome sequence and transcriptome of pomegranate peel reveal the pathway of biosynthesis of ellagitannins in pomegranate, explained by Qin et al. (2017). As reviewed all through, researchers from different regions of the world have indicated that the pomegranate peel and its chemical constituents like punicalagin and ellagic acid are effectual in hindering vital pathways at various stages of carcinogenesis, as depicted in Fig. 10.2.

10.6 Toxicity and Stability of Pomegranate Peel

Previous in vivo as well as in vitro studies prove that there is no adverse toxicity pomegranate peel on mammals, and it was also shown that in Wistar mice the intraperitoneal (i.p.) LD_{50} of aqueous extract of peel is 1321 ± 15 mg/kg (Qnais et al. 2007). The comparative study of median lethal dose (LD_{50}) for pomegranate peel extract revealed that the oral LD_{50} was more than 5 g/kg body weight (b.w.) and the i.p. LD_{50} in rodents, such as rats and mice, were 217 mg/kg b.w. and 187 mg/kg b.w., respectively. A dosage of 600 mg/kg/day of pomegranate peel extract was a NOAEL (no observed effect level) identified through a protocol of subchronic administration

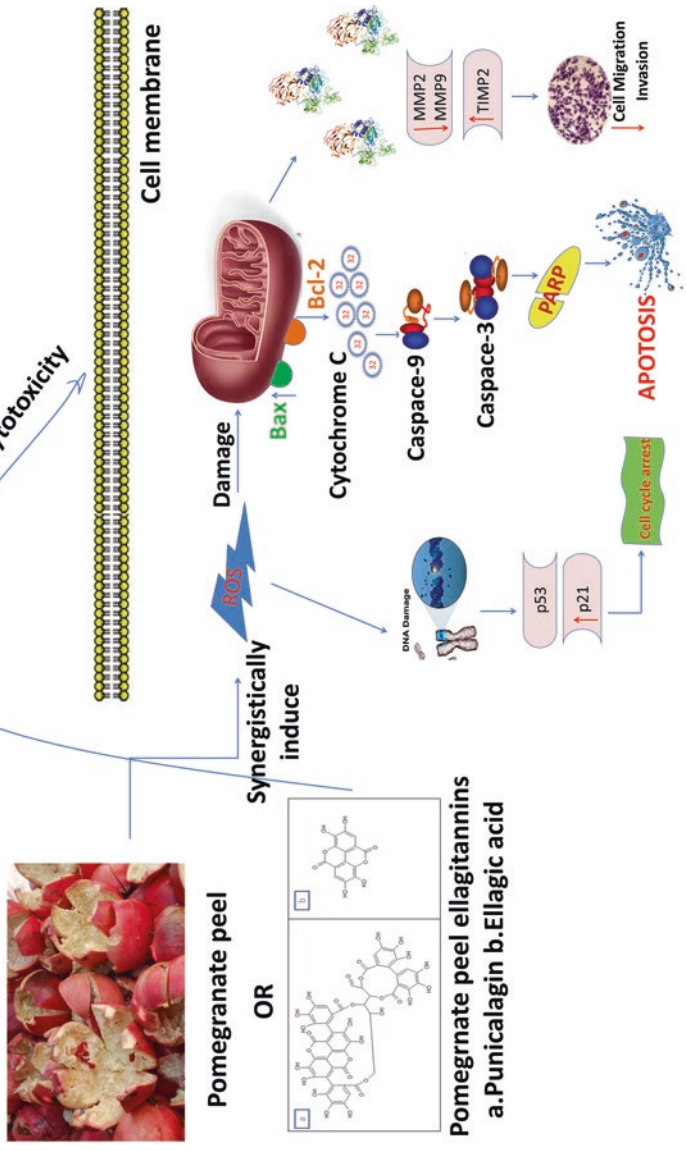

Fig. 10.2 Overall mechanisms of action of the pomegranate peel and its constituents

for a 90-day period (Patel et al. 2008; Eleonora et al. 2015). The first evidence of the possible genotoxicity of pomegranate peel used in folk medicine indicated no visible mutagenicity at the dose of 1–3 g/kg b.d. for 3 days in bone marrow cells (Sanchez-Lamar et al. 2008). The liquid extract of pomegranate peel was found to be stable at high temperatures of sterilization and at cold storage temperatures (Qu et al. 2013).

10.7 Clinical Studies

Around 13 clinical trials can be accessed from https://clinicaltrials.gov/ search page using the search term "pomegranate and cancer." One of the clinical trials was terminated for different reasons, six have been completed, three of each have been inactive, nonrecruiting, and of unknown status. Clinical trials with pomegranate peel in cancer are greatly lacking, despite the impressive amount of in vitro and preclinical studies, revealing its anticancer activity with no visible toxicity.

10.8 Conclusions

In the present scenario, employing dietary agents or functional foods for the prevention of cancer is a promising arena of oncology. The complementary and alternative medicine has drawn the attention of both clinical scientists and the general public due to dietary agents like pomegranate fruit and its waste product peel, having verified with their ability to avert or restrain cancers, their low cost, and trouble-free availability. Nevertheless, the present challenge lies in establishing the key compound or constituent of these functional foods liable for the anticancer consequences and the systems through which they stifle cancers. Scientific explorations grant ample amount of substantiations related to the bioactivities of pomegranate peel and its derivatives (products) with a focus on their anticancer features. Reports suggest promising chemopreventive/chemotherapeutic agents in pomegranate peel by exerting antioxidant, antiproliferative, anti-apoptotic, antimutagenic, and antitumorigenic effects by mitochondrial signaling pathway modulations. A significant amount of research reveals the in vitro efficiency of pomegranate peel against malignancy and promotion of cancer; however, in vivo and human trials are essential to authenticate the independent or existing therapies combined with the use of pomegranate peel against various cancers, such as breast, hepatic, prostate, skin, and thyroid cancers. It is anticipated that the chapter will provide inputs for the scientific community on the ongoing and further experimentations on pomegranate peel in cancer studies.

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Lycopene: Chemistry, Biosynthesis, Health Benefits and Nutraceutical Applications

11

Shubhi Singh and Smriti Gaur

Contents

Abstract

Lycopene is the natural colored pigment, and is known to impart red color to many fruits and vegetables. In tomatoes, lycopene is found at higher levels, but is also found in guavas, watermelons, papayas, mangoes, etc. Lycopene is the tetraterpene carotenoid compound, which is made up from the eight units of isoprene. These are also present as the active component in photosynthetic organisms and majorly their teleology is to help during important functions like photosynthesis and to provide defense to the host organisms from the overabundant light damage. This chapter discusses the biosynthesis pathway of lycopene, which has been formed from the precursor compound, isopentenyl pyrophosphate through mevalonic acid pathway. Apart from this, the synthesis of lycopene using the biotechnological strategies to increase its production has also been mentioned. Lycopene

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is considered as an option to lower the risk of several cancers like skin cancer, colon cancer, and prostate cancer, and it is associated with lowering the risks of the cardiovascular diseases, which have been discussed in detail too. This is considered as a very strong antioxidant; therefore, it protects the cells from the action of reactive oxygen species and prevents the cell damage. Studies show that these compounds are naturally present in *trans* forms and can change to *cis* forms on exposure to the abiotic stimuli. Lycopene also exhibits nutraceutical properties. For instance, it has been used as a lycopene-rich ice cream, a functional food that increases its bioavailability in the consumer's body. Besides many uses of this natural compound, future prospects must include more studies of its molecular mechanisms, finding of new potential therapeutic applications, and searching for the other ways to boost its production to meet increasing demand, hence helping to understand lycopene in an explicit and a better way.

Keywords
Terpenes · Isopentenyl pyrophosphate · Antioxidant · Medicinal · Health benefits

11.1 Introduction

Carotenoids are the important pigments present in plants. These are very essential for maintaining the plant health as well as imparting different colors to the plants like red, yellow, orange, etc., and these beautiful colors help the plants to attract many pollinators. Apart from this, the carotenoids are also responsible for absorption of light during photosynthesis (Hashimoto et al. 2016). These natural plant pigments not only are responsible to maintain the plant health, but are also known to exhibit several human health benefits. Two most important carotenoids are lutein and lycopene. Lutein is known to give yellow color to plants and belongs to category of xanthophylls. While, lycopene imparts red color to the plants and comes under the category of carotenes. Lycopene is abundantly present in tomatoes and tomato-related products, but can also be found in other fruits like apricots, watermelons, pink guava, etc., in small quantities. Studies were conducted to check the lycopene concentration in the food products, and it was observed that all tomatoes and the related products contain 0.8–94 mg/100 g of lycopene, whereas the concentration was reported to be 4.77 mg/100 g in watermelons, 8–18 mg/100 g in guavas, and 0.75 mg/100 g in grape fruits (Rao et al. 2018). In addition to these two, there are some other important bioactive carotenoids that are also present in plants like α carotenes, β carotenes, and γ carotenes, which come under the category of carotenes, and zeaxanthin which lies under the group of xanthophylls. Both α and β carotenes are known as the early precursors of Vitamin A synthesis. Zeaxanthin is known for its great antioxidative properties, and hence it reduces oxidation of many biomolecules (Langi et al. 2018).

Studies on lycopene and its various roles in benefiting the human health have drawn a great attention by researchers. Advancements in technology and more developments in research sector have clarified that lycopene is a potent antioxidant and prevents various kinds of cancers, cardiovascular health, diabetes, etc (Story et al. 2010). Various studies have been done on lycopene consumption worldwide, and it was stated that lycopene is primarily used not only as a health stimulant, but also as an additive in food industries (Kong et al. 2010). Generally, the naturally occurring state of lycopene is *trans* form, which provides much stability to the compound. All *trans* form consists of the open chain of hydrocarbons. But the working procedures like cutting, peeling, chopping, and crushing of the lycopene rich fruits can lead to its structural changes. Besides these mechanical factors, exposure to abiotic factors such as light, inappropriate pH, and high temperatures can lead to instability of its structure (Nikolova and Prokopov 2013). These factors result in the rotation of any of the bond present in lycopene structure. Because of this rotation, there is a conversion of all *trans* form to *cis* form, hence affecting the stability (Gupta et al. 2010). This chapter discusses the general chemistry of the compound, lycopene, the pathways involved in its synthesis, its role in benefiting the human health, and mechanisms of action. Simultaneously, this chapter also discusses various kinds of functional foods, which have lycopene as the major bioactive component.

11.2 Chemistry of Lycopene

Lycopene is the important compound from the family of carotenoid pigments. As discussed, carotenoid pigments can impart various colors to the plants. This family includes two major groups—Lutein and Lycopene. Lycopene is responsible for imparting the red color to the fruits and vegetables. The carotenoids are also known to play a very vital role in metabolizing Vitamin A and other metabolites. These carotenoids either have only hydrogen and carbons in their structures, known as hydrocarbon carotenoids, or have oxygen, hydrogen, and carbon in their structures, known as xanthophyll carotenoids (Story et al. 2010). The chemistry of lycopene is of great significance, as this compound is known to show the potential health benefits when it is present in its active form. The structure and chemistry of lycopene define how actively it will go and react to the free radical species and lower the effect of the oxidative stress. Lycopene is defined as the noncyclic carotenoid compound as it has 11 double bonds which are linearly arranged, and are conjugated in nature (Bunghez et al. 2011). Since this component is linear, it does not contain any ring structure, i.e., ionone ring. Because of this fact, the lycopene does not help in the metabolism of Vitamin A. The molecular formula of this acyclic compound is $C_{40}H_{56}$. Lycopene chemical structure is very sensitive to adverse abiotic conditions like high temperature, high pH, or even under light-stress conditions. Because of these abiotic factors, there are lots of changes occurring in the lycopene structure. The *trans* form of lycopene changes to *cis* form of lycopene, and hence affects its bioavailability inside the human body, and therefore exhibits the greater health benefits to the host. It was stated that *trans* form usually exists

in nature but the *cis* form was more stable. The *cis* form is known to contain lower melting point, more solubility in oils, and has no chances of crystallization and hence these forms of lycopene have more bioavailability in humans (Srivastava and Srivastava 2015). More recently, Shi and Maguer (2019) have mentioned that oxidation and isomerization are the two major reasons to change the beneficial properties of lycopene. These factors not only affect the nutrition content, but also change the color-imparting properties of this compound. They have also mentioned that by increasing the temperature to certain extent, the loss of lycopene in the products was observed. The study was also conducted where there was an increase in temperature from 90 to 150 °C, and hence isomerization and loss of lycopene was noticed after treating the tomato samples for a defined particular time. Along with these above-mentioned factors, the lycopene structure can get damaged from the non-thermal parameters also. These non-thermal operations on the tomato samples include cutting, grinding, peeling, or squashing. It was found that inappropriate peeling of the tomato samples can result in maximum loss of lycopene. Since lycopene is predominantly present in the tomatoes skin, peeling gives a maximum loss of this component (Martínez-Hernández et al. 2016). As already mentioned above, apart from isomerization, lycopene degradation can also be caused by the oxidation processes. In a study, it was found that cutting can induce more oxidation of lycopene. It was proved that cutting of tomato samples results in more activity of an important enzyme, lipoxygenase. As a result, it causes more oxidation of lycopene, and hence degradation of this bioactive compound occurs (Angaman et al. 2014).

11.3 Biosynthesis of Lycopene

All carotenoids are synthesized by plants and their synthesis is greatly increased when plants are provided with enough of sunlight and temperature. Plants having fruits with more bright colors are generally observed in areas of appropriate sunlight and temperature, since more number of carotenoids are present. Whereas in other icy places, plant fruits have some different colors as no enough carotenoids are being synthesized (Srivastava and Srivastava 2015). Lycopene is not only helpful for enhancing the human health, but also helpful to plants itself. It is known to protect the plant from light damage (Leong et al. 2018). It was also stated that upon UV light exposure, there is a formation of many reactive oxygen species and these are known to cause the damage to plant. Hence, carotenoids, especially the lycopene are known to act as a light-absorbing unit, and hence protect the damage of the plants as it lowers down the chances of formation of reactive oxygen species (Korkina et al. 2018). There are two main pathways involved in its synthesis—mevalonic acid pathway and non-mevalonic acid pathway. The main progenitor molecules for synthesis of tetraterpenes are IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate) (Cho et al. 2017). Mevalonic acid pathway start from the initial molecule acetyl CoA, which is further converted to acetoacetyl CoA by an enzyme Acetyl CoA acyltransferase. This molecule is then condensed to

HMG-CoA by an enzyme HMG CoA synthase, and then it again produces mevalonic acid. After the mevalonic acid is formed, the series of phosphorylation and decarboxylation reactions form the progenitor molecule—IPP (isopentenyl diphosphate). The key enzymes involved in this step are kinases and decarboxylases. This mevalonic acid pathway usually takes place inside cytosol and endoplasmic reticulum of plant cells (Singh and Sharma 2015). On the other side, non-mevalonic acid pathway starts with the initial molecule of pyruvate. Later, this pyruvate forms IPP and DMAPP through a series of conversions (Abdallah and Quax 2017). These IPP and DMAPP are 5-carbon molecules, and further these two molecules are condensed together to form 10-carbon molecule, called GPP (geranyl pyrophosphate). Then, more IPP is condensed to GPP to form 15-carbon molecule, called FPP (feransyl pyrophosphate). Further, IPP is condensed to FPP to form GGPP (geranylgeranyl pyrophosphate). The tetraterpenes are formed by condensation of 2 molecules of GGPP, shown in Fig. 11.1 (Wang et al. 2017). These pathways are naturally opted by plants to synthesize lycopene.

Except from these two pathways, there are some biotechnological strategies to enhance the production of lycopene. For instance, *Saccharomyces cerevisiae* is used as a model organism for enhancing the production of the lycopene. Since this

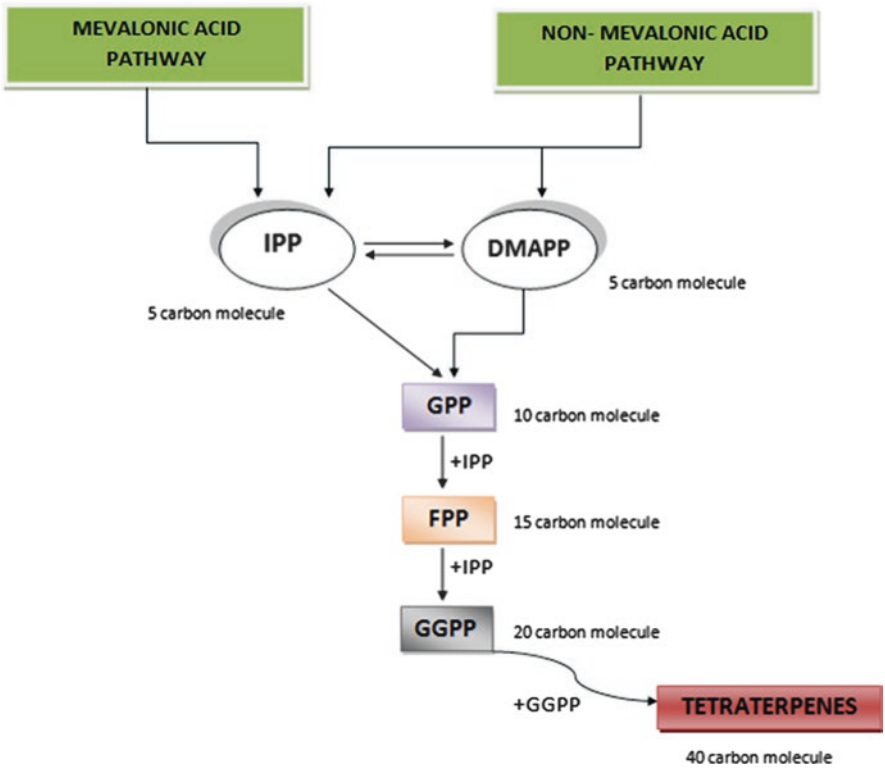

Fig. 11.1 Biosynthetic pathway of tetraterpenes

organism is considered to have the important initial molecules for terpenoids synthesis, the terpenoids synthesis pathway was engineered in this model organism using biotechnological techniques (Li et al. 2019). Lycopene production is increased not only by altering the synthesis pathway in any organism, but also by changing the cell membrane's ability to accumulate more of the produced lycopene. It was reported that genes responsible for more membrane bending were altered in *Escherichia coli* in addition to altered synthesis pathway. Because of this, more lycopene can get accumulated, and hence increasing its production (Wu et al. 2018). Besides these two organisms, another yeast model was also used for the same purpose. For instance, *Yarrowia lipolytica* was also engineered to enhance the lycopene production (Schwartz et al. 2017).

11.4 Health Benefits of Lycopene

There are many reported health benefits of lycopene. With increasing interest in this red colored pigment, more and more studies are still going on. This compound is known to exhibit the properties like lowering the risks of certain cancers, cardiovascular diseases, skin aging, diabetes, etc (Bhowmik et al. 2012). The detailed mechanism of its action has been described in the following sections.

11.4.1 Lycopene and Skin Aging

This may be defined as changes in the skin with time due to some external and internal factors. These changes in skin can be because of the diseases, exposure to UV rays, exposure to pollutants, diet of the host, lifestyle, alcohol consumption, and many more (Aseervatham et al. 2013). Exposure to above-mentioned factors leads to the formation of ROS (reactive oxygen species). Mitochondria of the cell are known to be responsible for the production of these ROS, such as O_2^- (superoxide anion), OH (hydroxyl radical), and H_2O_2. These ROS are further stabilized by an important enzyme, superoxide dismutase (SOD). Once stabilized ROS are formed, these are released from mitochondria, and then these go and bind to DNA, other proteins and lipids, and hence cause the cell damage. Because of this damage, the important adhesion proteins like collagen and elastin get damaged, and hence we see changes in our skin (Cui et al. 2012). Hence, to stop these oxidative stress reactions, we must take antioxidants. These antioxidants will not only prevent our cells from getting damaged, but also will heal the defective tissues. Lycopene is known to be a potent antioxidant, which prevents the damage of skin cells from oxidative stress. This component is also known to provide collagen and elastin, which in turn reduces the chance of getting wrinkles and maintains a healthy skin (Salavkar et al. 2011). As bioavailability of lycopene is the major issue, hence more advanced research led to the discoveries of nanoemulsions. These nanoemulsions carry our bioactive compound lycopene and release it at the required place. One such study describes that lycopene-loaded nanoemulsions were made with defined size, and hence this increased bioavailability of lycopene with more potent antioxidative property

(Ganesan and Choi 2016). Apart from the nanoemulsions, the lycopene-supplemented food is also another way for releasing the bioactive compound in the host with the aim of maximizing bioavailability.

11.4.2 Lycopene and Cancer

The statistical data for 2018 reported that cancer has become one of the deadly diseases in the world. These data state that around 9.6 million people die because of this disease. It was also mentioned in these data that people suffer largely from lung cancer, followed by breast cancer and then prostate cancer (Bray et al. 2018). Lycopene has been reported as a potent bioactive compound for lowering the risk of cancer in humans. As discussed above, lycopene is able to decrease the effect of ROS, and hence prevents the damage. In relation to cancer, studies were done to explain that lycopene is able to prevent the process of cancer cells formation. This bioactive component is able to arrest the G1 phase of cell cycle of cancer cells, and hence results in their reduced cellular proliferation (Palozza et al. 2011). It was also mentioned that many lung cancers develop, because of smoking cigarettes. This cigarette smoke is known to increase the levels of ROS in the lungs. Thus, there will be damages to proteins, lipids, and DNA of the cells. Again, this damage is considered to increase the rate of cancer cell formation (Valavanidis et al. 2013). Hence, lycopene was proved to counter this mechanism. Lycopene is known to increase the formation of phase II detoxifying enzymes. These enzymes increase the antioxidative function, and therefore prevent the cancer cell formation (Wang 2012). Apart from the lung cancer prevention, lycopene has also been studied in relation to breast cancers. A study demonstrates that lycopene is known to lower down the metastatic growth of the cancer cells, and hence inhibits the proliferation. Lycopene is considered to inhibit the important signaling pathway of tumor development like MAPK/Erk pathway and PI3K/Akt pathway (Ko and Moon 2015). Uppala et al. (2013) have reported that lycopene is able to prevent the phosphorylation of certain molecules, such as Cytokeratin 19. This molecule plays a major role in growth of the cells. Lycopene has also been reported to inhibit the phosphorylation of Cytokeratin 19 at position Serine-35. Also, this potent bioactive component is known to enhance the activity of an important enzyme, α Enolase as this enzyme acts as a tumor suppressor in nature and hence prevents the cancer-like conditions. Gap junctions are very important for cell-to-cell communication. Healthy cells have better gap junctions, and hence cells remain in healthy conditions. Connexins are known to play a very important role in GJC. But in cancer-like conditions, gap junction communications are not very well maintained. Lycopene is known to enhance the expression of connexins genes so that easy communication is achieved between the cells, and hence cells remain in healthy state (Sahin et al. 2015). Lycopene is also known to inhibit the formation of pro-inflammatory compounds. For instance, a study proved that lycopene is able to stimulate the immune response as more number of CD4+ and CD8+ cells were produced. Apart from this, the pro-inflammatory compound numbers were also decreased, which proved that the immunity was enhanced during

cancer-like conditions (Chai et al. 2017). The overview of lycopene action in prevention of cancer has been given in Fig. 11.2.

11.4.3 Lycopene and Cardiovascular Diseases

Cardiovascular diseases also have become the major cause of deaths in the past few years. Cardiovascular diseases are known as the disease associated with heart and the blood vessels, which carry blood and oxygen. The main causes for the occurrence of cardiovascular disease are considered to be stress, unhealthy diet, poor lifestyle, smoking, overweight, age, lack of exercises, pollution, etc. The main reason for the occurrence of such conditions is the accumulation of bad cholesterol, LDL cholesterol. This LDL cholesterol has an outer shell or covering of phospholipids and proteins like Apolipoprotein (ApoB). This ApoB carries and helps the LDL cholesterol to attach to the walls of the arteries by the ionic interactions. Once the interaction is done, the accumulation of LDL starts, and hence this accumulation blocks the oxygen and blood flow, causing heart diseases (Hansson and Hermansson 2011). Lycopene is known to reduce the accumulation of these molecules in the arterial tract, and thus enhances the easy flow of oxygen and blood. Lycopene tends to lower down the adhesion of such molecules and hence stops the stiffness of arteries (Mozos et al. 2018). It is also known that once the attachment of LDL cholesterol is completed, the oxidation of this bad cholesterol starts. This oxidation is initiated by many enzymes or by ROS, and hence this causes damage to the arteries where the cholesterol is attached. As reported, enzymes like NADPH oxidase and

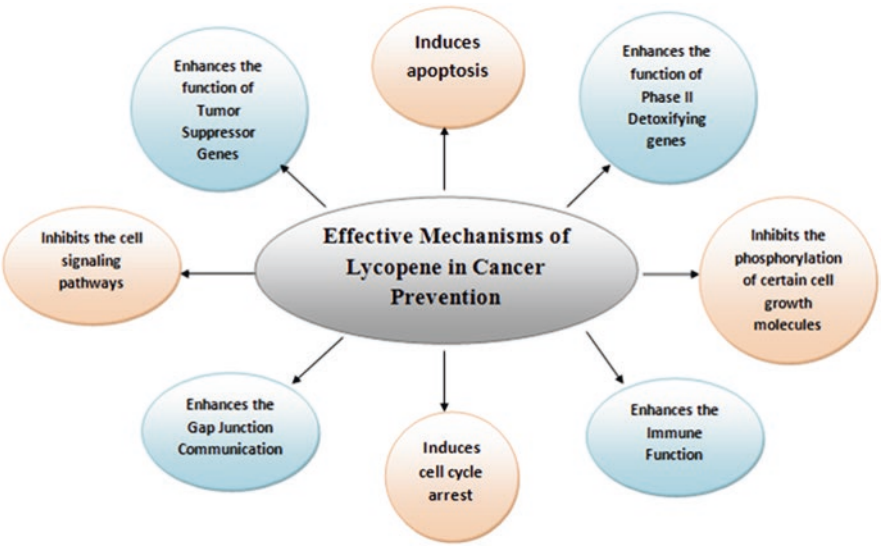

Fig. 11.2 Effective mechanisms of lycopene in cancer prevention

myeloperoxidase are known to cause the oxidation. Apart from these enzymes, ROS are also involved in the same function (Peluso et al. 2012). Lycopene is known to attenuate the oxidation of LDL cholesterol, and hence mitigates the damage caused to the arteries (Assis et al. 2017). Another known reason for the occurrence of cardiovascular diseases is the low levels of HDL cholesterol. This HDL cholesterol is considered to be good for health. Lycopene is considered to maintain and increase the levels of good cholesterol in the blood. This means that chances of cellular damage and arteries blockage significantly decrease (Bohm 2012).

11.4.4 Lycopene and Diabetes

Globally, diabetes has become so common nowadays, and the recent data states that its spread is increasing at a very high rate. This is a disease in which patient's blood glucose levels fluctuate from the normal levels. There is a very important enzyme called glucokinase, which acts as a detector to detect how high the blood glucose levels have gone in the human body and accordingly the insulin is released. But in diabetic patients, this enzyme is known to get dysfunctional. As a result, there is no automatic system in our body to detect high blood glucose levels, and hence appropriate insulin is not released to counter the effect (Grewal et al. 2014). Lycopene is known to be beneficial in case of diabetes too. It was reported that lycopene is considered to enhance the activity of glucokinase enzyme, and as a result the blood glucose levels are maintained at the desired levels (Eze et al. 2016). Lycopene is also known to lower the risk of diabetes as this bioactive compound helps in lowering the blood lipid levels (Yin et al. 2019).

11.4.5 Other Health Benefits

Apart from the above-mentioned health benefits, lycopene is also known to increase the bone mass. It was reported that lycopene-induced rats were found to have much stronger bones with high bone mass and good architecture (Ardawi et al. 2016). Also, lycopene is known to have a direct link with the kidneys and the disease associated with kidneys. Due to obesity, chances of renal damage become high. This is because of the fact that during obesity, the inflammatory responses are also increased (Ellulu et al. 2017). These inflammatory responses are known to cause the renal damage. When lycopene was tested, it was proved that it attenuates the level of inflammatory responses, and therefore lowers down the chances of damage to kidneys (Pierine et al. 2014). It was also reported that lycopene is very effective in lowering down the neuropathic pain. Neuropathic pain generally occurs when the nerves and neuronal tissues get damaged. The nitric oxide and TNF-α are known to increase the inflammation, and hence cause the damage to nearby nerves and tissues. As a result of this inflammation, pain arises. Lycopene is known to lower down the release of TNF-α as well as nitric oxide, and hence relieves the pain (Lim and Kim 2016). In addition to these inflammatory factors, neuropathic pain is also

caused by the increased expression of the connexins, which in turn leads to the release of many cytokines, and hence increases the amount of gap junctions between the tissues (Morioka et al. 2019). Lycopene has been reported to maintain proper functioning of the gap junctions, and hence attenuate the pain. It has also been stated that lycopene can cross the blood–brain barrier very easily. Due to this fact, it can directly prompt the central nervous system to function in a positive way. In addition to this, this bioactive component is also known for its great antioxidative nature. Lycopene is best known for rummaging the free radicals. Because of this property, it lowers down the effect of oxidation of many natural occurring molecules, and hence helps in the treatment of Parkinson disease and Alzheimer disease (Malekiyan et al. 2019). Besides these health benefits, lycopene has a great potential in supporting the eye health. Its antioxidative property also prevents the damage of the optic tissues and the optic nerves.

11.5 Lycopene in Functional Foods

With the advancement in technology, lycopene is being used up in different kinds of food. Nowadays, people are becoming more health conscious, and hence they are easily accepting functional foods. Lycopene-rich ice creams have recently come up in the market. In these ice creams, lycopene have been supplemented in the ice cream to increase its bioavailability. It was seen that this ice cream was able to show the antioxidative property, and was able to reduce the acne occurrence on the skin in the consumers (Chernyshova et al. 2019). Apart from this, lycopene from dried tomato waste was added during the preparation of breads. Later, when it was analyzed, this was concluded that bread was able to show antioxidant property and had better characteristic properties as compared to normal breads (Nour et al. 2015). Formulations in different types of oils have been made too. Various types of oils have been enriched with lycopene, for instance, walnut oil has been enriched with lycopene at a defined concentration. This formulation has led to the changes in its constituent's profile. The levels of the saturated and unsaturated fatty acids were reported to have changed, the phenolic content levels were also stated to have fluctuated, and the antioxidant property was reported to have enhanced. Overall, the shelf life and the quality of this formulated walnut oil were enhanced (Xie et al. 2018). Lycopene-rich beef hamburgers were made to intensify the texture and overall quality of the burgers (García et al. 2009). Daily consumable snacks, when supplemented with lycopene promise a very good option for dealing with hunger pangs in terms of both taste and quality. Wojtowicz et al. (2018) have made a study of developing the lycopene-rich corn snack. It was observed that addition of dried tomato lycopene up to certain level provided the good texture results, but beyond that it was not acceptable. Also, bioavailability of this bioactive compound was enhanced, hence it making it a promising functional food. Another study reported that lycopene was used as a replacement of fat component in sausages. The tomato peels were dried and were made into powder. Later, this tomato powder was incorporated in the recipe of sausages instead of other animal fats. The results confirmed

the good texture, color, and minimized fat content and hence making it a better alternative for consumption (Wang et al. 2016).

11.6 Conclusions

This chapter describes the chemistry, biosynthesis, and the important health benefits of the natural red-colored pigment of plants, lycopene. This compound has received the most attention from research point of view. This has shown the great positive health benefits in cell culture experiments or during animal studies. But, mechanisms involved behind its action are still needed to be explored. The most important fact which must be considered is that its bioavailability varies from person to person. This depends upon many factors like lifestyle, age, health of the person, metabolism, lycopene intake, etc. As a result of this, the bioactivity of this compound differs. Further, in future, studies need to be done to check whether the effect of lycopene is alone or it shows some synergistic effect with other molecules. Apart from its mechanism of action, the clear metabolic fate of lycopene still needs to be researched more. Besides many stated health benefits like prevention of cancer, cardiovascular diseases, diabetes and many more, future researches must be continued to check its other health benefits. In addition to this, new ways of biotechnological synthesis must be invented so that more and more lycopene production continues in future to meet the increasing demand. Being a new alternative in the field of medical sciences and the food industry, lycopene plays a crucial role in maintaining good lifestyle. It has been seen as a possible solution to perpetuate health. New insights into the pioneering of lycopene-based functional foods and their utility assure the great promise only by the prolonged researches on this bioactive compound.

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12 Dietary Agents in the Prevention of Cataractogenesis: Results from Preclinical Observations

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Contents

Abstract

Cataract formation is one of the foremost reasons of blindness, especially in the elderly people. The process is shown to be hastened by aldose reductase (AR)

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enzyme involved in catalyzing the reduction of wide-ranging aldehydes to their corresponding alcohols. Gene mutations are connected to secondary cataract formation in the aged population. Further, the age-linked cataract formation is also due to the oxidative stresses. The scientific experiments conducted with preclinical models of studies have shown that dietary agents and phytochemicals are effective in reducing and mitigating cataractogenesis by acting as inhibitors of AR, preventing the depletion of antioxidant enzymes, inhibiting lipid peroxidation, and reducing oxidative stresses. This chapter addresses the helpful properties of phytochemicals in inhibiting AR. Also, the other mechanisms of action of phytochemicals in mitigating the cataract formation are highlighted.

Keywords
Aldose reductase · Indian gooseberry · Cataractogenesis · Lemon · Grapes · Sweet orange · Litchi · Mangosteen

12.1 Introduction

Among various sense organs of the body, the eye and its function of visual perception are of utmost importance in one's day-to-day life (Bourne et al. 2017). Humans are very unique in their dependency on eyesight as the foremost sense, and this is reflected in the complexity of our eyes as compared to other animals. The lack of sight or low vision not only impairs one physically, but also has psychological, social, and economic implications (Bourne et al. 2017). Cataract is a term used to denote any variation in its refractive index or opacity in the crystalline lens. The cataract formation is the foremost reason for visual impairment or lack of vision globally (Hobbs and Bernstein 2014; Braakhuis et al. 2019). The global incidence of blindness in 2015 was about 36 million, and it was estimated that another 0.217 billion populace live with the modest-to-severe blindness. Totally, 0.253 billion people were reported with blindness as recorded by a survey in the year 2015 (Bourne et al. 2017). The WHO (World Health Organisation) has designated cataract as the foremost basis of vision impairment, accounting for nearly 50% (17 million) of the incidence of vision loss worldwide (Bourne et al. 2017). With the increase in population and life expectancy, this number is only going to rise in the days to come (Fletcher 2010; Weikel et al. 2014; Braakhuis et al. 2019). As described earlier, the number of cataract cases in the world is predicted to be nearly 40 million by the year 2020 (Sowmya et al. 2015). Further, estimates are that by the end of 2050, approximately two billion people worldwide will be more than 60 years of age. Among them, closely 0.039 billion will be completely visionless and 0.246 billion will grieve from imperfect eyesight (Braakhuis et al. 2019). This will considerably hinder the growth and development of people, their families, and further the nation's economy at a large scale.

The prevalence of cataract in India was observed to be about 70%. The incidence of non-operated cataract was found to increase with the time of life, and observed to be greater in females than in menfolk as reported by Sowmya et al. (2015). The frequency of non-operated cataract in individuals, who are above 60 years of age was found to be 58% in the northern part of India, while it was 53% in the southern parts of India (Vashist et al. 2011). It is also noted to be a major cause of visual impairment in lower socioeconomic population. This could be explained by the lack of accessibilities to medical facilities, poor diet, and increased exposure to sunlight, pollutants, and other environmental factors (Vashist et al. 2011). It continues to remain as one of the topmost source of vision loss in low-income and middle-income nations, due to lack of availability of healthcare resources in these countries (Sowmya et al. 2015). Furthermore, facts also specify that approximately 90% of the world's visual impaired people live in emerging nations, where medicinal amenities are not as the best in comparison to the advanced nations. The ratio of ophthalmologists to patients is quite scarce to cater to the eye care needs of the population suffering from eye ailments. This disparity can only be sorted out by emphasizing on the need for preventive methodologies in tackling certain eye ailments and slowing down the disease progression. Presently, a number of cataract surgeries are done worldwide. However, the cost involved in surgeries is very expensive, and is burdening the nations. It was estimated that more than 30 million US $ was paid out on civic inpatients for cataract operations, and postsurgery treatment services during the period from 2009 to 2010 (Braakhuis et al. 2019). Alternatively, the better approach is to postpone the onset of cataract formation. The inception of cataract delayed by 10 years will shrink the incidence of cataractogenesis up to 50%. This will impressively reduce the expense associated with surgical treatments (Brian and Taylor 2001; Braakhuis et al. 2019). As cataract formation is a progressive illness of aged population, any effective therapeutic cure should be formulated. In this direction, it is likely that dietary supplements or herbal products can be the best treatment when delivered either through eye drops or orally. There are several reports to support that the dietary supplements, such as the Indian gooseberry, turmeric, orange, mangosteen, lemon, grape, litchi, blue berry, finger millet, sickle senna, and their bioactive compounds, can prevent or reduce the cataractogenesis (Li and Jiang 2007; Choudhary and Gulia 2011; Puppala et al. 2012; Shobana et al. 2013; Devi et al. 2014; Ha et al. 2014; D'souza et al. 2014; Ma et al. 2014; Chaudhury et al. 2015; Abengózar-Vela et al. 2015; Sreelakshmi and Abraham 2016a, 2016b; Andjelic et al. 2017; Crespo and Visioli 2017; Braakhuis et al. 2019). Plant-derived flavonoids like genistein, quercitrin, isoflavone, etc. have shown to delay the diabetic cataractogenesis. Some examples of plant products having AR inhibitory property are the extracts of ethnic herbs, such as *Withania somnifera, Ocimum sanctum, Azadirachta indica, Curcuma longa* or diabecon (the Indian herbal formulation) (Huang et al. 2007; Pollreisz and Schmidt-Erfurth 2010). This chapter provides a comprehensive data on the etiology of cataractogenesis, and the role of phytocompounds and their mode of actions in the inhibition of cataract formation.

12.2 About Cataract

Cataract is a malady of eyes, where the lens of eyes becomes cloudy leading to vision loss. Cataract formation is very commonly expected, and linked to the aging process. Normally, the lens of eye is very clear and it focuses incoming light rays onto the light-sensitive tissues, retina present at the backside of the eye. To have a perfect appearance onto the retina, the eye regions facing forward to the retina, comprising the lens must be transparent and clear. When the light strikes the eye, a chemical reaction is initiated within the retina leading to induce electrical signal, which is carried to the brain through the optic nerves. Later, the brain will determine what is observed by eyes. When the lenses are normal and clear, the retina will receive a sharp and clear image. However, in a cataract eye, the lens cloudy and gives a blurry vision. Further, the degree of the optical disruption is reliant on the level of cloudiness of the lens.

Cataract can be classified as congenital, age-related or secondary due to some medical illnesses like diabetes (Braakhuis et al. 2019). Age-linked cataract is the most frequent one, which is responsible for nearly half the blindness worldwide. The lens is clear when one is born and continues to be so till sometime after the age of 45, after which progressive opacities begin to form, initiating the process of cataractogenesis. The major 3 subtypes of age-related cataracts include nuclear, posterior, subcapsular, and cortical cataracts. The genetic predisposition, oxidative stress, UV radiation, and calcium-level abnormalities area few of the causative factors that are believed to aid in the process of cataractogenesis (Sowmya et al. 2015).

Mutations in the EPH2A gene coding for tyrosine kinase have been known to be connected with age-related cortical cataract (Shiels et al. 2008; Jun et al. 2009). The lens epithelium, being the anterior portion of the lens, is greatly vulnerable to the effects of UV radiation and reactive oxygen species (ROS)-mediated oxidative stresses. It is believed that they bring about a decrease in the cell density of epithelial cells by inducing apoptosis. This in turn increases p53expression, thereby decreasing the levels of soluble sulfhydryls, glutathione reductase, catalase, and superoxide dismutase (Johar et al. 2003). It is also believed that with aging, an internal barrier to antioxidants develops, following which the crystallin protein forms in the center of the lens nucleus. Hence, it becomes susceptible to the attachment of reactive molecules and oxidation, resulting in protein cross-links and denaturation, glycation, and progressive hardening of the nucleus (Truscott 2005). This is often accompanied by color discoloration of the lens, wherein it turns yellow to brown, and subsequently to black with a progressive hardening.

Consistently, high blood sugar levels or diabetes is also a potential and leading cause for cataract development in affected individuals. It is believed that AR, a key Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent enzyme in the polyol pathway, which results in converting glucose into sorbitol is associated with hyperglycemic cataractogenesis (Pollreisz and Schmidt-Erfurth 2010). This was proven by the disproportionate accretion of sorbitol observed intracellularly in the diabetic animal models. This emphasizes the role of using AR inhibitors in the prevention of cataractogenesis (Reddy et al. 1984; Andjelic et al. 2017). In addition to

this, calcium plays a vital part in maintaining the homeostasis of lens. It is assumed that in cortical cataract, the intracellular homeostasis is disrupted, leading to abnormal intracellular levels of calcium that exceeds the lens' ability to remove calcium from the cytosol. This in turn triggers the breakdown of structural proteins, and eventually causes the cell death (Duncan and Jacob 1984; Gosak et al. 2015).

The progression of cataract is reliant on a number of factors. Till date, we have only a limited understanding on the underlying mechanisms of cataractogenesis. Ongoing research and investigation of the process of cataractogenesis are of paramount importance, due to the magnitude prevalence of this condition worldwide. Cataracts are frequently measured to be an inevitable result of aging. Although surgery is the mainstay of its management at present, the modern etiological investigations have recognized the mediations that may possibly thwart or delay the cataractogenesis process. Several efforts have been engaged in delaying the inception and retardation of cataracts progression using different types of inhibitory agents. Due to its primary role in cataractogenesis, AR is one the main target molecules, which can help in preventing the progression of cataractogenesis. For that reason, efforts are being carried out in developing pharmacological agents that are precise and effective in inhibiting AR action. Gene mutations are also linked to secondary cataract formation in the aged population. For instance, a mutation in the gene galactokinase 1 (GALK1) that encodes the enzyme in galactose metabolism is triggered to form hypergalactosemia and cataract. Likewise, the age-linked cataract formation is also due to the oxidative stresses (Tewari et al. 2019). The search for an optimal synthetic agent is still a continuously ongoing process. In this direction, phytotherapy has been successfully proven to be very effective, and considered widely, due to the fact that it is less toxic and free from side effects. Hence, this has compelled the requirement of potential preventive agents, especially the dietary phytochemicals. Several promising research works have revealed that few plants and their phytochemicals could be beneficial (Majumdar and Srirangam 2010; Liu et al. 2017; Tewari et al. 2019). To substantiate these, seminal case–control studies by Tavani and coworkers in 1996 have shown that diet rich in butter, total fat, and salt increases risks, while regular consumption of cheese, meat, cruciferae, tomatoes, spinach, peppers, melon, and citrus fruits shows beneficial effects. Also, several plant bioactive compounds are effective in preventing or reducing cataractogenesis (Tavani et al. 1996). In the subsequent sections, the beneficial effects of plants and their bioactive compounds in overcoming the cataractogenesis are addressed.

12.3 Plants and Their Phytochemicals Against Cataract Formation

12.3.1 Indian Gooseberry

Indian gooseberry (*Phyllanthus emblica*), belonging to the family *Euphorbiaceae* is also known as Amla. It is one among the highly valuable curative herbs used in the

Indian traditional medicinal systems, such as the Ayurveda, Unani, and Siddha (Variya et al. 2016; Husain et al. 2019). Their fruits are also identified as the myrobalans or berries, and are the main portion of Amla used in food preparations and medicinal applications. Indian gooseberry fruits are a chief dietary agents, and hence utilized in making murabbah, ladu, burfi, fresh juice, chutneys, pickles, and curries in India (Baliga and Dsouza 2011; Thilakchand et al. 2013; D'souza et al. 2014). Amla fruits are extensively used in several countries of the Southeast Asia as traditional medicines. They are used in treating illnesses, such as diabetes, asthma, cough, bronchitis, ophthalmopathy, cephalalgia, erysipelas, hemorrhoids, skin diseases, nervine debility, inflammation, leprosy, dyspepsia, emaciation, colic, flatulence, peptic ulcer, hyper-acidity, jaundice, dysentery, diarrhea, hemorrhages, menorrhagia, leucorrhoea, cardiac disorders, anemia, intermittent fevers, liver complaints, jaundice, leucorrhea, menorrhagia, osteoporosis, hematuria, inflammation of the eyes, and weak vision (Krishnaveni and Mirunalini 2010; Baliga and Dsouza 2011; Thilakchand et al. 2013; D'souza et al. 2014; Variya et al. 2016).

Indian gooseberry and its phytocompounds are reported to be effective in preventing cataractogenesis. Indian gooseberry aqueous extract was reported to inhibit the recombinant human AR with an IC_{50} value of 0. 88 mg/ml. They found that aqueous extract containing tannoids is the main phytoconstituents accountable for the inhibition of AR (Suryanarayana et al. 2004). Later, a group of researchers investigated the role of Indian gooseberry tannoids in inhibiting cataract formation (Suryanarayana et al. 2007). They found that STZ (streptozotocin)-induced diabetic rats with cataract when treated with Indian gooseberry enriched with tannoids significantly reduced cataractogenesis. Research investigations have identified1-O-galloyl-β-D-glucose (β-glucogallin) as the chief phytocompounds of fruits, and it is shown to be very effective in the selective inhibition of Aldo-ketoreductase family 1member B1 (AKR1B1) genes under in vitro conditions (Puppala et al. 2012). Further, molecular modeling investigations have shown that β-glucogallin effectively binds to the enzyme, AR, at its active site and arbitrates the AR inhibitory effects. The successive studies also confirmed the role of β-glucogallin in the inhibition of sorbitol accretion up to 73% when treated at the concentration of 30 μM under hyperglycemia state observed in the organ culture model (ex vivo), involving transgenic mice lenses with overexpressing human AR in the lens (Puppala et al. 2012).

12.3.2 *Curcuma longa*

Curcumin, the bioactive phenolic constituent of turmeric (*Curcuma longa* L.) is reported to exhibit antioxidative and hypoglycemic properties as confirmed by in vitro and in vivo studies (Suryanarayana et al. 2003; Suryanarayana et al. 2005; Kumar et al. 2005; Raju et al. 2006; Manikandan et al. 2011; Grama et al. 2013; Kocaadam and Şanlier 2017). It is a widely used as spice in many cuisines. It is traded as a cosmetic ingredient, herbal supplement, food colorant, and food flavoring agent (Kocaadam and Şanlier 2017). It was seen that lenses collected from rats

treated with curcumin for 2 weeks (75 mg/kg/day) were more resistant to 4-HNE (4- hydroxy 2- transnonenal) induced opacification of lenses compared to control animals (Suryanarayana et al 2003). It seems to especially play a role in preventing galactose-induced cataractogenesis. Its protective role in ionizing radiation-induced cataractogenesis has also been studied by Ozgen et al. (2012), wherein cataract formation was observed to be 100% in the irradiated groups, and the rate of cataract reduced to 40% in the curcumin treated groups. The treatment of curcumin was observed to improve the levels of vitamin C, and thus involve in preventing cataract progression (Murugan and Pari 2006). Curcumin was reported to repress Hsp 70, αA-crystalline, and αB-crystallin expressions in STZ-prompted cataracts in animal model (Kumar et al. 2005; Manikandan et al. 2011). The chief mechanism involved in the cataract inhibition by curcumin is reported to be due to antioxidant properties (Manikandan et al. 2009; Manikandan et al. 2010; Manikandan et al. 2011; Radha et al. 2012). Likewise, Chhunchha et al. (2011) have reported that curcumin can hinder pleiotropic oxidative stress-related proteins, Prdx6 (peroxiredoxin 6) in hLECs (human lens epithelial) cells. A study by Cao et al. (2018) proposed that curcumin can attenuate selenite-prompted cataract by reducing the intracellular secretion of reactive oxygen species.

12.3.3 Grape

Grapes, scientifically known as *Vitis vinifera,* is globally one of the most important fruits with immense dietary, medicinal, and financial uses. The juice of grapes is being used throughout the world for its effective curative values, comprising ocular-promoting activity. Its beneficial uses are accredited to the occurrence of phytocompounds like resveratrol, procyanidins, or proanthocyanidins, which are chemically the dimers, trimers, and oligomers of monomeric epicatechins or catechins in them (Singleton 1992; Bartolome et al. 1996). Studies have shown that phytocompounds occurring in grape juice have been linked to the regulation of glucose metabolism, maintenance of intraocular pressure, and destruction of proinflammatory cytokines in the system (Natarajan et al. 2017). Reports suggest that grape phytochemicals mitigate several ocular problems, including uvea, macular degeneration, cataractogenesis, diabetic retinopathy, and red eyes (Natarajan et al. 2017). Reports suggest that resveratrol, a principal constituent of grape, is reported to be beneficial on cataracts, and other ocular diseases (Natarajan et al. 2017; Goutham et al. 2017).

Resveratrol treated to IOBA-NHC (human conjunctival) and HCE (corneal) epithelial cells was effective in mitigating the TNF-α or ultraviolet (UVB) radiation induced secretion of interleukin (IL)-6, IL-8, and Interferon gamma-induced protein 10 (IP-10) in a dose-dependent manner (Abengózar-Vela et al. 2015). The combination of quercetin and resveratrol was observed to enhance their protective properties indicating that these polyphenols may have a therapeutic potential in overcoming inflammatory ocular surface diseases (Abengózar-Vela et al. 2015). Mechanistic studies indicate that the beneficial effects of resveratrol are facilitated by antioxidant, antiapoptotic, antitumorigenic, antiinflammatory, anti-angiogenic,

and vasorelaxant properties (Bola et al. 2014). In addition to resveratrol, studies have also shown that the grape polyphenols (MGPs) were effective in reducing ocular inflammations and Endoplasmic Reticulum (ER) stresses.

Seminal studies by Ha et al. (2014) with cultured ARPE-19 (human retinal pigmented epithelium) cells and common inbred strain of laboratory mice (C57BL/6) have demonstrated that grape seed polyphenols effectively attenuate ER stresses and ocular inflammations. The investigators observed that treatment of ARPE-19 cells with grape seed polyphenols reduced the tumor necrosis factor (TNF)-α-encouraged proinflammatory gene expression of monocyte chemotactic protein-1, IL-1β, and IL-6 by reducing the activation of mitogen-activated protein kinase (MAPK), and subsequently reducing the levels of activated nuclear factor κ-B (Ha et al. 2014). Experiments also showed that grape seed polyphenols were effective in mitigating the thapsigargin-mediated ER stress in ARPE-19 cells. Further, studies with C57BL/6 mice showed that treating with grape seed polyphenols reduces the leukocyte infiltration and acute ocular inflammations. Mechanistic observations revealed that grape seed polyphenols reduce inflammation-intervened loss of tight junctions and retinal permeability (Ha et al. 2014). The grape seed procyanidins and other antioxidative phytocompounds were effective in thwarting the development of cataractogenesis via their antioxidative activities (Yamakoshi et al. 2002). Moreover, quercetin (a flavonoid) occurring in grapes is shown to decrease oxidative responses as well as inflammations in human ocular surface epithelial cells (Abengózar-Vela et al. 2015). Cumulatively, all these studies clearly showed that treatment with grape seed polyphenols reduced ER stress-related vascular endothelial growth factor (VEGF) secretion, unfolded protein responses, and early apoptosis to mediate its protective effects (Ha et al. 2014).

12.3.4 *Cassia tora*

Cassia tora Linn., colloquially known as the sicklepod plant, commonly found in India and other tropical countries, is known for many medicinal importance (Jain and Patil 2010; Choudhary and Gulia 2011). It is found growing wild in low-lying coastal area, on riverbanks, and in waste land. The plant is known as "Chakramard" in Ayurveda, "Panwar" in Unani, and "Jue Ming Zi" in the Chinese system of medicine. The plant is utilized in various traditional systems of medicines as a laxative, antiseptic, antioxidant, and antiperiodic, and is reported to be useful in leprosy, ringworm, bronchitis, cardiac diseases, hepatic disorder, liver tonic, hemorrhoids, and ophthalmic and skin ailments (Jain and Patil 2010; Choudhary and Gulia 2011). The leaves have been shown to be rich in polyphenols, emodin, quercetin, kaempferol-2-diglucoside, chrysophanol, aloe-emodin, rhein, glucose, 1-stachydnine, amino acids, fatty acids, d-mannitol, β-sitosterol, myricyl alcohol, trigonelline, choline, sennosides, and ononitol monohydrate (Jain and Patil 2010; Choudhary and Gulia 2011).

With regard to its protective effects on the eye, studies by Sreelakshmi and Abraham (2016a) and Sreelakshmi and Abraham (2016b) have observed that

feeding *C. tora* leaves effectively mitigated selenite-influenced cataract formation in rat pups by restoring membrane integrity, antioxidant levels, downregulating epithelial cell death, and by reducing the accumulation metal. Further, the extract also seemed to be effective in prevention of cataract by maintaining lens architecture. From a phytochemical perspective, these effects seem to be mediated by the polyphenols, such as chrysophanol, stigmasterol, kaempferol, emodin, quercetin, and isoquercetin present in leaves (Sreelakshmi and Abraham 2016a; Sreelakshmi and Abraham 2016b). Likewise, previous studies have also shown quercetin as an effective agent in mitigating the oxidative and inflammatory responses in human ocular surface epithelial cells (Abengózar-Vela et al. 2015).

12.3.5 Tea

Globally, green tea is highly preferred as a beverage, and is reported to be helpful in preventing/moderating various illnesses. Traditionally, this beverage used in the form of green tea in China, and even today, it is extensively consumed in several countries (Jigisha et al. 2012). Phytochemical studies have confirmed the myriad of medicinal values of tea, especially credited to their phytoconstituents, such as epicatechin, catechins, epigallocatechin, epigallocatechin-3-gallate, epicatechin-3-gallate, proanthocyanidins, quercetin, myricetin, kaempferol, and gallic acids. These green tea polyphenols are reported to have antioxidant, anticancer, and anti-inflammatory properties, and hence are beneficial for the eyes as well (Ma et al. 2014; Thichanpiang and Wongprasert 2015; Chaudhury et al. 2015).

Scientific investigations have revealed that the principal phytochemical epigallocatechin-3-gallate was effective in preventing the alteration of intact tryptophan residues in γ-crystallin protein isolated from cataractous human eye lens (Chaudhury et al. 2015). Further, the pretreatment of cultured ARPE-19 cells with epigallocatechin-3-gallate (EGCG) caused a decrease in the TNF-α-induced increase of intracellular ROS (Thichanpiang and Wongprasert 2015). EGCG also ameliorated the inflammatory effects of TNF-α as observed by the increase in the degree of monocyte-retinal pigment epithelium adhesion, and concomitantly decreased upregulation of ICAM-1 (Intercellular Adhesion Molecule 1), the nuclear translocation of phosphor-NF-κB, and decreased the expression of phosphor-NF-κB and IκB degradation (Thichanpiang and Wongprasert 2015). Together, all these observations affirm that EGCG ameliorated the inflammatory effects by suppressing the TNF-α signaling by inhibiting the NF-κB pathway. Animal study results have revealed that application of tea polyphenol gel was effective in protecting the lens epithelial cells in rabbits subjected to vitrectomy (Ma et al. 2014). The investigators performed unilateral vitrectomy with silicone oil tamponade on 2-month-old New Zealand white rabbits. The tea polyphenol gel was applied topically in the surgical eyes and the animals were sequentially sacrificed on days 45 and 90 post-operation (Ma et al. 2014). The eyes were harvested and quantified for the levels of ROS, mitochondrial membrane potential ($\Delta\Psi m$), and apoptosis of lens epithelial cells (Ma et al. 2014). The outcomes of this research further confirmed that the

application of the tea polyphenol gel reduced the generation of ROS, maintained ΔΨm, inhibited the overexpression of caspase-3, and decreased apoptosis of lens epithelial cells.

12.3.6 Finger Millet

Eleusine coracana (Finger millet) is globally an essential grain, and widely cultivated in the tropical parts of South Asia and Africa. Among millets, finger millet ranks the fourth position after sorghum, pearl millet, and foxtail millet. According to some estimates, nearly three million tons of finger millet are produced annually (Shobana et al. 2013; Devi et al. 2014). Finger millets are rich in calcium, dietary fiber, phytates, protein, minerals, iron, phenolics, riboflavin, thiamine, methionine, phenylalanine, leucine, isoleucine, and other essential amino acids. Phytochemical studies have shown that finger millets also contain ferulic acid, *p*-coumaric acid, proto-catechuic acid, gallic acid, p-hydroxybenzoic acid, cinnamic acid, syringic acid, trans-cinnamic acid, ferulic acid, quercetin, kaempferol, phloroglucinol, naringenin, luteolin, apigenin, epicatechin, catechin, catechin gallates, trimers, and tetramers of catechin and proanthocyanidins. However, their concentration varies with the geographic and temperature conditions (Shobana et al. 2013; Devi et al. 2014). From a health perspective, consumption of finger millets is known to possess antidiabetic (type 2 diabetes mellitus), antidiarrheal, antiulcer, antiinflammatory, antitumorigenic (K562 chronic myeloid leukemia), antiatherosclerogenic effects, antimicrobial, and antioxidative properties.

With regard to the beneficial effects of finger millet in cataractogenesis, Chethan et al. (2008) investigated the inhibitory effects of the important finger millet phytochemicals like gallic acid, protocatechuic acid, p-coumaric acid, 4-Hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, and trans-cinnamic acids. They found that quercetin inhibited the activity of AR in cell-free assay. Further, investigations have reported that quercetin was effective in decreasing the inflammatory and oxidative responses in human ocular surface epithelial cells (Abengózar-Vela et al. 2015), while luteolin was effective in reducing inflammation in human retinal pigment epithelial cells (Hytti et al. 2017). The results suggest that the finger millet seed coat polyphenols were effective in inhibiting AR reversibly by noncompetitive inhibition making it a potential dietary agent in hindering cataract formation in humans (Chethan et al. 2008).

12.3.7 Blueberries

Blueberries (*Vaccinium* sp.) are originally an American fruit species that are found growing well in the humid woodlands. Classified in the family Ericaceae, blueberries consist of approximately 450 species. Blueberries contain vitamin C, procyanidins, kaempferol, quercetin, myricetin, hydroxycinnamic, stilbenes, ellagic acid, and anthocyanins like malvidin, delphinidin, petunidin, cyaniding, and peonidin

(Johnson and Arjmandi 2013; Crespo and Visioli 2017). Practically, blueberry fruit's outer layer is the most valuable part, as it comprises closely all of the anthocyanins. Since antiquity, blueberries are being used in our diet; however, their health benefits are understood very recently, and still researches are being undertaken continuously. Scientific studies carried out in accordance with pharmacological guidelines have shown that blue berries possess antioxidant, antiinflammatory, anticancer, neuroprotective, and cardioprotective effects (Crespo and Visioli 2017).

Experiments by Liu et al. (2015) have shown that the flavonoid-rich fraction was effective in thwarting visible light-tempted docosahexaenoic acid lipid peroxidation compared to the phenolic acid- and anthocyanin-rich fractions (Liu et al. 2015). Rutin, quercetin glycosides, isoquercetin, chlorogenic acid, and hyperoside present in blueberry are also effective in preventing cataractogenesis in vivo and in vitro (Ferlemi et al. 2016). Additionally, quercetin is shown to reduce the oxidative responses and inflammation in human ocular surface epithelial cells (Abengózar-Vela et al. 2015). However, cyanidin-3-glucoside and quercetin were shown to safeguard the pigmented layer of retina against photooxidation and photodegradation (Wang et al. 2017). Together, all these observations indicate the usefulness of blueberries in eye care.

12.3.8 Litchi

Litchi chinensis (Litchi), belonging to the family, *Sapindaceae* is an important dietary agent. Historical evidence indicates that the plant was originally indigenous in the geographical region of Southern China. However, today the plant is also cultivated on a large scale in semi-tropical areas of the world (Li and Jiang 2007). Litchi is a well-investigated plant and chemical studies have confirmed that they contain flavanols like procyanidin B2, procyanidin B4, and epicatechin. Also, litchi contains anthocyanins like cyanidin-3-glucoside, cyanindin-3-rutinside, quercetin-3-glucoside, and quercetin-3-rutinosde. However, their ratio is dependent on the climate and soil conditions (Li and Jiang 2007). Scientific experiments have confirmed that these phytochemicals possess myriad pharmacological effects and that the antioxidant activity is responsible for the beneficial effects at least in part (Li and Jiang 2007). Experimental studies with laboratory rat lenses have confirmed that the extracts of the litchi fruits exhibit inhibitory effects on cataract formation. Also the methanol extract and ethyl acetate fractions were shown to be the most effective. Cell-free studies with the isolated phytochemicals have shown that delphinidin 3-O-β-galactopyranoside-39-O-beta-glucopyranoside showed the highest AR enzyme inhibitory activity with an IC_{50} value of 0. 23 μg/ml, indicating its possible uses and further exploration in the prevention of cataractogenesis.

12.4 Conclusions

Preclinical examinations performed in the recent past have affirmatively confirmed that many dietary agents and phytochemicals are beneficial in inhibiting/preventing/retarding the process of cataractogenesis by mediating myriad pathways. However, a major drawback is that many of these observations are from cell-free and in vitro assays and have not been studied involving animal models. On a brighter side, the fact that a large number of dietary agents and phytochemicals are beneficial is of interest, as this gives a choice for clinical studies in the future and paves way for industrial use of the phytochemicals. However, the factors that need to be considered here are toxicity profile (drug safety), cost factors, availability, convenience of administration, and acceptance. However, the fact that these agents are of dietary origin have wide acceptability, and possess myriad health benefits (Van Duyn and Pivonka 2000) is the driving source for future animal and clinical studies that need to be undertaken and affirmed for the benefit of especially the high-risk group of people prone to cataractogenesis.

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Bioactive Xanthones from *Garcinia mangostana*

13

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Abstract

Garcinia mangostana Linn. (mangosteen) is a tropical plant, widely cultivated in Asia including Malaysia, Thailand, Indonesia and India. The pericarp of *G. mangostana* has been used traditionally to treat skin infections, wounds, dysentery, urinary disorders, cystitis and gonorrhoea. Claiming for its benefits in promoting health, mangosteen fruit juice is listed as one of the bestselling supplements worldwide. There are over 60 xanthones together with benzophenones and flavonoids that have been identified in the aerial parts of *G. mangostana*. Among the bioactives, xanthones are known to exhibit intriguing pharmacological activities including anti-cancer, anti-inflammation, anti-viral, anti-diabetic and anti-neurodegenerative properties. These medicinal effects are explored in greater detail to elucidate their potential uses as a therapeutic agent. This chapter presents an updated information on *G. mangostana* derivatives and their pharmacological effects and mechanisms of action against chronic diseases.

Keywords

G. mangostana · Bioactives · Xanthones · Pharmacological effects

13.1 Introduction

Garcinia mangostana is a tropical plant belonging to the family, *Clusiaceae*. It is believed that *G. mangostana* is an allotetraploid derived from *G. hombroniana* Pierre and *G. malaccensis* T. Anderson (Richards 1990; Lim 2012). The *G. mangostana* trees can grow up to 25 m in height. The leaves are dark green with glossy texture on the surface and yellowish beneath (Morton 1987) (Fig. 13.1). As tropical climate is required for the growth, *G. mangostana* is widely cultivated in the tropical countries of Southeast Asia (as shown in Fig. 13.2). Mangosteen, the fruit of *G. mangostana* is also known as the ‘queen of the fruits’. This edible fruit consists of

Fig. 13.1 *G. mangostana* leaf and unripe fruit (left), whole tree (middle) and fruit (right)

soft and juicy white internal pulp with a dark purple rind, and has a sweet taste and pleasant aroma.

Different parts of the *G. mangostana* tree have been used traditionally for various medicinal purposes. For example, the decoction of the *G. mangostana* pericarp was used as folk medicines to treat a wide array of diseases, such as diarrhoea, skin diseases, inflammation, cholera, wounds, urinary disorder and amoebic dysentery (Garnett and Sturton 1932; Chopra et al. 1956; Mahabusarakam and Wiriyachitra 1987; Pierce 2003). In countries, such as the Philippines and Malaysia, the leaves and bark decoction have been traditionally used as a febrifuge to treat fever, thrush, diarrhoea, dysentery and urinary disorder. The root decoction was consumed by women to regulate the menstrual cycle and ease menstrual pain (Lim 2012). Meanwhile, the bark and young leaves were employed to combat diarrhoea, dysentery and genital-urinary tract infections in India (Burkill 1966; Perry 1980; Lim 2012). In Indonesia, the mangosteen leaves were used for the wound recovery, especially after circumcision (Lim 2012).

To date, six clinical studies have been carried out to investigate the health potential of the mangosteen extract, including atrial fibrillation, chronic periodontitis and weight loss (http://www.clinicaltrials.gov). It is worth noting that no clinical study has been conducted on the mangosteen compounds. This chapter provides an update of the secondary metabolites of *G. mangostana* and the pharmacological effects of these bioactive compounds against emerging diseases including cancer, diabetes, influenza, neurodegenerative disorders, tuberculosis, as well as their anti-inflammatory potential.

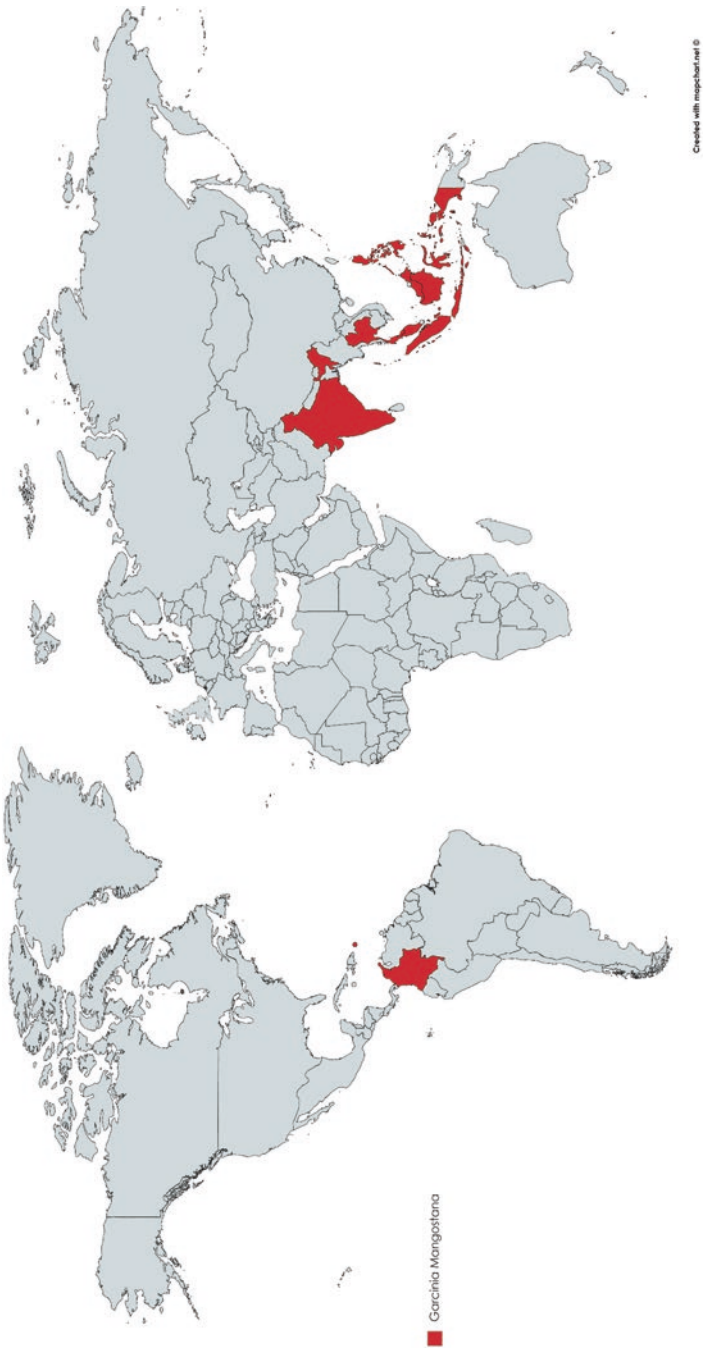

Fig. 13.2 The geographical distribution of *G. mangostana*

13.2 Extraction and Isolation of Xanthones from *G. mangostana*

Medicinal plants serve as a valuable reservoir of novel molecules with therapeutic potential as more than half of the clinically approved chemical entities in recent decades are derived from plants (Atanasov et al. 2015). Nevertheless, it is estimated that only a small portion of the molecules have been investigated phytochemically and pharmacologically for their therapeutic potential (Hostettmann and Wolfender 2000). The bioactive compounds derived from plants are classified into different classes based on their chemical structures, functions and biosynthesis origin. The major classes of phytocompounds include terpenes, flavonoids, saponins, alkaloids, coumarins and phenolic compounds.

G. mangostana is rich in phenolic compounds including xanthones, flavonoids and benzophenones. The majority of the compounds found in *G. mangostana* are prenylated and oxygenated xanthones. More than 60 naturally occurring xanthones have been purified from different parts of the plant including fruit hull, fruit, leaves and bark. Various conventional means have been used to retrieve secondary metabolites from a complex matrix of plant material, including maceration and soxhlet with some shortcomings, such as generation of hazardous organic wastes and degradation of heat labile compounds. Hence, cost-effective and efficient means are imperatively in need to replace the conventional extraction methods. Microwave-assisted extraction, enzyme-assisted extraction and supercritical fluid extraction are among the greener technologies with a shorter extraction time, and use of minimum or no organic solvent. Efficient and environment-friendly extraction methods have been applied in the extraction of compounds of *G. mangostana*, mainly α-mangostin. For example, Ghasemzadeh and colleagues demonstrated that the recovery of α-mangostin concentration in fruit hull by microwave-assisted extraction was 72.40% (v/v), with the extraction time of 3.16 min and microwave power of 189.2 W (Ghasemzadeh et al. 2018). The amount of α-mangostin in mangosteen pericarp was found to be 121.01 mg/g dry matter. Supercritical fluid extraction is another environmentally benign technology used for the extraction of α-mangostin from the fruit hull of mangosteen. The optimum conditions for the recovery of α-mangostin (yield 0.2% w/w) were 100 bar, 140 °C, extraction time of 1 h (Chhouk et al. 2016).

13.3 Biological Activities

Plants have been documented for their medical uses in treating illnesses in humankind. These medicinal plants have played significant and beneficial roles in drug discovery as they produce novel pharmacologically active compounds with unique and diverse structures (Khazir et al. 2014; de Oliveira Júnior et al. 2018). Several medicinal values of the extracts and compounds derived from *G. mangostana* have been determined, including anti-cancer, anti-inflammatory, anti-diabetic, anti-influenza and anti-neurodegenerative properties.

13.3.1 Anti-cancer Activity

Plant-derived secondary metabolites are the promising source of chemotherapeutic drugs (Khazir et al. 2014). These compounds are now being used as cancer therapeutics due to their ease of availability and cost-effectiveness (Kuppusamy et al. 2013). Almost half of the anti-cancer drugs are natural product-based compounds (Newman and Cragg 2015). The examples of plant-derived chemotherapeutic agents available for clinical use are vinca alkaloids (vinblastine and vincristine) from *Catharanthus roseus* (Blaskó and Cordell 1990), paclitaxel from the bark of *Taxus brevifolia* (Rowinsky and Donehower 1995) and camptothecin from *Camptotheca acuminata* (Wall et al. 1966). Many plants are still actively investigated for their potential for cancer treatment.

To date, more than seven xanthones including α-mangostin, β-mangostin, γ-mangostin, garcixanthone B, garcixanthone C, mangostinone, garcinone E and 2-isoprenyl-1,4-dihydroxy-3-methoxyxanthone have been isolated from different parts of *G. mangostana*, and studied extensively for their anti-cancer activities. Several research findings have revealed that these xanthones possess a broad spectrum of anti-cancer properties in a variety of cancers, such as breast cancer, colorectal cancer, leukaemia, gastric cancer, pancreatic cancer and liver cancer. Table 13.1 summarises some of the recent in vitro anti-cancer properties of the xanthones with their respective mechanisms of action.

Most of the anti-cancer effects of xanthones are contributed by their ability to inhibit cell proliferation, induce apoptosis and promote cell cycle arrest. Induction of apoptosis serves as one of the therapeutic strategies in cancer treatment (Igney and Krammer 2002). Apoptosis is described as programmed cell death, which maintains the physiological balance between cell death and survival in a multicellular organism. It involves complex molecular signalling mechanisms which lead to the changes in the nuclear morphology (including pyknosis and karyorrhexis), the formation of apoptotic bodies, blebbing of the plasma membrane and cell shrinkage (Kroemer et al. 2009). Apoptosis is the preferred mechanism in cancer treatment as compared to other types of cell death (necrosis and autophagy) as it possesses the advantage of the elimination of cells without causing inflammation (Festjens et al. 2006).

The molecular targets modulated by the xanthones on different signalling pathways for the anti-cancer effect are summarised in Fig. 13.3. Among the xanthones, α- and γ-mangostin are particularly highly cytotoxic by exerting apoptotic effect through intrinsic or mitochondrial pathway through up-regulation of the expression of pro-apoptotic Bcl-2-associated X protein (BAX) and activation of cysteine-dependent aspartate-directed proteases (caspase) such as caspase-3 and -9 (Krajarng et al. 2011; Chen et al. 2014; Kritsanawong et al. 2016). These xanthones also induced apoptosis via extrinsic or death receptor pathway with the activation of caspase-8, which further activated the cleavage of BH3 interacting domain death agonist (Bid) (Watanapokasin et al. 2011). Cancerous cell proliferation was inhibited through mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and human epidermal growth factor receptor 2 (HER2) signalling

Table 13.1 Pharmacological effect and mechanisms of action of compounds derived from *G. mangostana*

Compound	Type of cell	Pharmacological effect	Mechanisms of action	References
Bone cancer				
α-Mangostin	SW 1353	IC_{50} value of 10 μg/mL (24 h)	Induction of apoptosis through intrinsic pathway Inhibition of cell proliferation through MAPK and ERK pathway	Krajarng et al. (2011)
Breast cancer				
α-Mangostin	BT 474	IC_{50} values of 2.91 μM (24 h), 2.28 μM (48 h) and 3.15 μM (72 h)	Cell cycle arrest at G1 phase	Ittiudomrak et al. (2018)
	T47D	IC_{50} value of 7.5 μM (24 h)	Induction of apoptosis through intrinsic pathway with up-regulation of activated caspase-9 and Bax/Bcl-2 ratio	Kritsanawong et al. (2016)
	COLO205	IC_{50} value of 9.74 μM (24 h)	Induction of apoptosis through extrinsic pathway with activation of caspase-8 and t-Bid	Watanapokasin et al. (2011)
	MCF-7	IC_{50} values of 3.57 μM (24 h) and 2.74 μM (48 h)	Induction of apoptosis with up-regulation of cleaved PARP	Li et al. (2014)
	MDA-MB-231	IC_{50} values of 3.35 μM (24 h) and 2.26 μM (48 h)	–	Li et al. (2014)
γ-Mangostin	MCF-7	IC_{50} value of 5.27 μM (24 h)		
Garcixanthones B		IC_{50} value of 4.27 μM (48 h)		Xu et al. (2014)
Garcixanthones C		IC_{50} value of 3.081 μM (48 h)		Ibrahim et al. (2018a, b)

(continued)

Table 13.1 (continued)

Compound	Type of cell	Pharmacological effect	Mechanisms of action	References
Colorectal cancer				
α-Mangostin γ-Mangostin	HCT116 and SW480	IC_{50} value of 15 μM (72 h)	Down-regulation of the expression of beta-catenin protein	Yoo et al. (2011)
α-Mangostin	DLD-1	IC_{50} value of 7.5 μM (24 h)	Cell cycle arrest at G1 phase; induction of apoptosis through intrinsic pathway	Akao et al. (2008)
β-Mangostin	DLD-1	IC_{50} value of 8.1 μM (24 h)	Cell cycle arrest at G1 phase	
γ-Mangostin	DLD-1	IC_{50} value of 7.1 μM (24 h)	Cell cycle arrest at S phase	
γ-Mangostin	HT-29	IC_{50} values of 51.87 μM (24 h), 69.15 μM (48 h) and 85.38 μM (72 h)	Induction of apoptosis via induction of intracellular ROS	Chang and Yang (2012)
Leukaemia				
α-Mangostin	K562	IC_{50} values of 16.35 μM (24 h), 13.8 μM (48 h) and 7.71 μM (72 h)	Cell cycle arrest at G1 phase with significant up-regulation of p21; Induction of apoptosis by up-regulation of cleaved caspase-3 and PARP	Chen et al. (2014)
	KBM5	IC_{50} values of 10.26 μM (24 h), 8.2 μM (48 h) and 6.13 μM (72 h)		
	KBM5-T315I	IC_{50} values of 10.57 μM (24 h), 7.08 μM (48 h) and 5.87 μM (72 h)		
	K-562	IC_{50} value of 7.21 μg/mL (24 h)	–	Novilla et al. (2016)
	Lymphocyte (human donor)	IC_{50} value of 25 μg/mL (24 h)		
	HL-60	IC_{50} value of 1.12 μg/mL (24 h)		
	HL-60	IC_{50} value of 6.8 μM (72 h)	Induction of apoptosis via activation of caspase-3	Matsumoto et al. (2003)

(continued)

Table 13.1 (continued)

Compound	Type of cell	Pharmacological effect	Mechanisms of action	References
β-Mangostin	HL-60	IC_{50} value of 7.6 μM (72 h)	–	Matsumoto et al. (2003)
γ-Mangostin	HL-60	IC_{50} value of 6.1 μM (72 h)		
Mangostinone	HL-60	IC_{50} value of 19 μM (72 h)		
Garcinone E	HL-60	IC_{50} value of 15 μM (72 h)		
2-Isoprenyl-1,4-dihydroxy-3-methoxyxanthone	HL-60	IC_{50} value of 23.6 μM (72 h)		
Stomach cancer				
α-Mangostin	BGC-823 and SGC-7901	–	Induction of apoptosis via inactivation of STAT3 signalling pathway	Shan et al. (2014)
Pancreatic cancer				
α-Mangostin	MIA PaCa-2	IC_{50} values of 8.4 μM (48 h) and 8.5 μM (72 h)	–	Kim et al. (2017)
	PANC-1	IC_{50} values of 15 μM (48 h) and 11.7 μM (72 h)	Induction of apoptosis with up-regulation of caspase-3, cleaved PARP and Bax	
	MIA PaCa-2	IC_{50} values of 15 μM (48 h) and 11.7 μM (72 h)		
γ-Mangostin	PANC-1	IC_{50} values of 25 μM (48 h) and 10.2 μM (72 h)		
Brain tumour				
γ-Mangostin	U87 MG	IC_{50} value of 74.14 μM (24 h)	–	Chang et al. (2010)
	GBM 8401	IC_{50} value of 64.67 μM (24 h)	Induction of apoptosis via production of ROS	Chang et al. (2010)

(continued)

Table 13.1 (continued)

Compound	Type of cell	Pharmacological effect	Mechanisms of action	References
Nasopharyngeal cancer				
γ-Mangostin	CNE1	IC_{50} value of 1.85 μM (24 h)	–	Xu et al. (2014)
	CNE2	IC_{50} value of 1.81 μM (24 h)		
	SUNE1	IC_{50} value of 4.41 μM (24 h)		
	HONE1	IC_{50} value of 2.78 μM (24 h)		
Lung cancer				
γ –Mangostin	A549	IC_{50} value of 3.79 μM (24 h)	–	Xu et al. (2014)
	GLC82	IC_{50} value of 3.46 μM (24 h)		Xu et al. (2014)
Garcixanthones B	A549	IC_{50} value of 2.65 μM (48 h)	–	Ibrahim et al. (2018a, b)
Garcixanthones C	A549	IC_{50} value of 3.91 μM (48 h)		

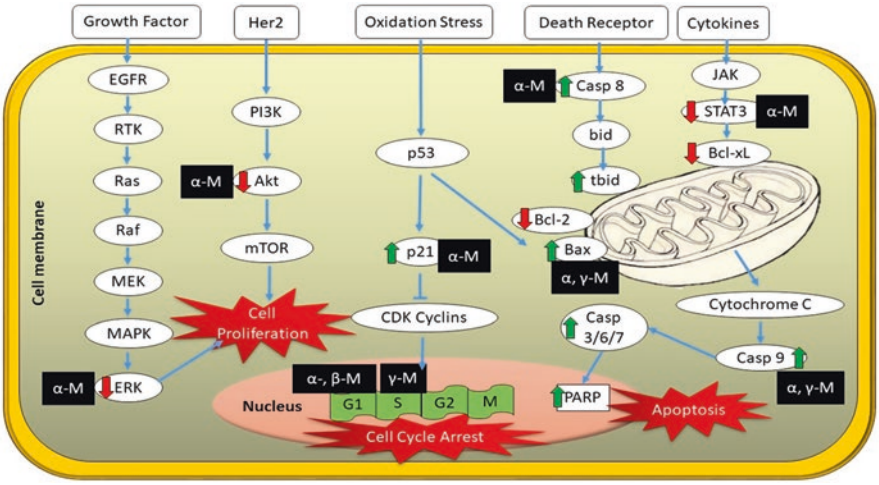

Fig. 13.3 Detailed mechanism of action and molecular targets modulated by the xanthones (α-, β- and γ-mangostin)

pathways by down-regulation of total and phosphorylated ERK1/2 and serine/threonine-protein kinase (Akt) after treatment with α-mangostin (Krajarng et al. 2011). Cell cycle arrest at the G_1 phase and S phase was induced by α- and γ-mangostin, respectively, by modulation of cyclin-dependent kinases (CDKs) and activation of p21 following reactive oxidative stress (ROS) (Akao et al. 2008; Ittiudomrak et al. 2018). From the literature, as most of the promising activities are mainly attributed

to α-mangostin, it is claimed that α-mangostin may act as a pleiotropic agent that targets multiple signalling pathways for its anti-cancer properties.

13.3.1.1 Against Lung Cancer

Lung cancer represents a serious health problem due to its high incidence with 2.094 million new cases diagnosed (about 11.6% of all cancers) and remains as the first cancer killer with 1.8 million deaths reported (approximately 18.4% of total cancer deaths) (Bray et al. 2018). Even with the advancement in the healthcare technology, the mortality and recurrence rate of this disease is still high, therefore urging the search for more efficient chemotherapeutic agents that would increase the survival and reduce the mortality rate of patients.

Zhang and colleagues isolated three xanthones (garcimangosxanthone A-C) to determine their cytotoxic effect against lung cancer cells. Garcimangosxanthone A and B exhibited promising in vitro cytotoxic assay against human alveolar basal epithelial cancerous cell A549 and human pulmonary adenocarcinoma cell LAC with half maximal inhibitory concentration (IC_{50}) values ranging from 5.7 to 25 μM after 72-h treatment (Zhang et al. 2010). Ibrahim and colleagues have demonstrated that garcixanthones B and C, the new xanthones extracted from the fruit pericarps, exhibited cytotoxic effects against human lung cancer cell A549 with IC_{50} values of 2.65 μM and 3.91 μM, respectively (Ibrahim et al. 2018a, b). Even though these xanthones have proven to exhibit high cytotoxicity against lung cancer cells, no study has been carried out to investigate the mechanism underlying the efficacy.

13.3.1.2 Against Breast Cancer

Breast cancer, the malignant tumour that forms in breast tissue, is the most common cancer diagnosed in women worldwide with an estimated 2.1 million new cases, representing 11.6% of total cancer diagnosed in 2018. It is the leading cause of death among women, with an estimated 0.7 million deaths, representing 6.6% of all cancer deaths (Bray et al. 2018). A number of studies proved that the xanthones, specifically α-mangostin, hold a great potential as the anti-breast cancer agent. It showed significant cytotoxicity against breast cancer cells with different molecular characteristics such as human breast ductal carcinoma BT474, oestrogen receptor (ER)-positive human breast carcinoma T47D, ER-positive human breast adenocarcinoma MCF-7 and ER-negative MDA-MB-231 cell lines with IC_{50} values less than 10 μM (Akao et al. 2008; Watanapokasin et al. 2011; Li et al. 2014; Ittiudomrak et al. 2018). Most of the studies have concluded that α-mangostin exerted its cytotoxicity by the induction of apoptosis through both intrinsic and extrinsic pathways and cell cycle arrest at the G_1 phase. Apart from α-mangostin, Xu et al. (2014) and Ibrahim et al. (2018a, b) demonstrated that γ-mangostin, garcixanthone B and C exhibited cytotoxicity against MCF-7 with IC_{50} values of 5.27, 4.27 and 3.08 μM, respectively.

Given the promising in vitro results, studies have been carried out to unveil the potential of xanthones in the in vivo breast cancer animal model. Shibata et al. (2011) demonstrated that oral administration of 20 mg/kg of α-mangostin has significantly increased the survival and suppressed the tumour growth of mouse

mammary BJMC3879luc2 model. This study showed that α-mangostin could be a potential anti-metastatic agent in which it significantly reduced the lymph node metastasis in the animal model. Another study carried out by Doi et al. (2009) revealed the potential of panaxanthone (consisting of 80% of α-mangostin and 20% of γ-mangostin) as an anti-metastatic agent against breast cancer with prominent suppression of the lung metastases in the BJMC3879 mouse model.

13.3.1.3 Against Colorectal Cancer

Colorectal cancer is the third most common cancer, with 1.8 million new cases being diagnosed worldwide (approximately 10.2% of the new cases reported). It is the second most common cause of cancer death after lung cancer with an estimated of 0.88 million of deaths reported (approximately 9.2% of all cancer deaths) (Bray et al. 2018). As the colorectal cancer is a slow progression disease (takes approximately 10–15 years to become invasive cancer), early diagnosis, screening and prevention are the keys to enhance the survival rate of the patients. With the limitations of screening tests and poor prognosis, research has been focused on chemoprevention properties to reduce the mortality rate (Yoo et al. 2011).

Yoo et al. (2011) revealed that the xanthones, specifically α- and γ-mangostin, could be potential chemoprotective agents for colorectal cancer by inhibiting the Wnt/β-catenin signalling pathway, a crucial part in the cancer development. The chemopreventive properties of α-mangostin are also proven by Akao et al. (2008), showing that dietary administration of α-mangostin (up to 0.05%) has significantly inhibited the development of aberrant crypt foci in the 1,2-dimethylhydrazine-induced rat model.

Apart from chemopreventive properties, some studies revealed the potency of xanthones as chemotherapeutic agents. Mangosteen-derived xanthones showed in vitro anti-cancer properties against human colorectal adenocarcinoma COLO 205 cell line by inhibition of cancerous cell proliferation and induction of cell death via apoptosis by activation of the caspase cascade. In vivo analysis using the COLO 205 tumour mouse model showed that the growth of tumours was repressed upon intra-tumoural administration of mangosteen xanthones at relatively low doses (0.25 mg per tumour).

13.3.1.4 Against Leukaemia

Leukaemia, a cancer of blood cells caused by abnormal proliferation of non-functional cells in the bone marrow, has been on the rise with 437,033 new cases reported with estimated 309,006 cancer deaths in 2018 (Bray et al. 2018). With the high mortality rate and relapsed upon treatment discontinuation, the search for an effective chemotherapeutic agent for leukaemia is required. Studies revealed that α-mangostin could be a potential anti-cancer agent for leukaemia as it selectively inhibited the proliferation and induction of apoptosis in human leukaemic cells including K562, KBM5, KBM5-T315I, HL60 and K-562 with minimal toxicity towards normal lymphocyte (Chen et al. 2014; Novilla et al. 2016). The α-mangostin induced apoptosis through up-regulation of cleaved caspase-3 and PARP and arrested cell cycle at G_1 phase with significant up-regulation of p21 (Chen et al.

2014). Other xanthones such as β-mangostin, γ-mangostin, mangostinone, garcinone E and 2-isoprenyl-1,4-dihydroxy-3-methoxyxanthone also exhibited cytotoxic effects towards HL-60 leukaemia cells with the IC_{50} values of 7.6, 6.1, 19, 15 and 23.6 μM, respectively, after 72-h treatment (Matsumoto et al. 2003).

13.3.1.5 Against Skin Cancer

Skin cancer, specifically malignant melanoma, is one of the major health problems with 287,723 cases reported and an estimated 60,712 deaths in 2018 (Bray et al. 2018). The cytotoxic effect of α-mangostin, γ-mangostin and 8-deoxygartanin was investigated on the human melanoma SK-MEL-28 cell line. The study revealed that γ-mangostin and 8-deoxygartanin at the concentration of 5 μg/mL increased cell cycle arrest at the G1 phase, while α-mangostin at a concentration of 7.5 μg/mL had the highest percentage of apoptotic cells (induced 59.6% early apoptosis). Also, α-mangostin induced apoptosis in SK-MEL-28 cell line via caspase activation (25-fold increase in caspase-3) and disruption of mitochondrial membrane potential (Wang et al. 2011).

13.3.2 Anti-inflammatory Activity

Pro-inflammatory cytokines play an essential role in inflammatory diseases. The overproduction of pro-inflammatory cytokines including tumour necrosis factor (TNF-α), interleukin-1-beta (IL-1β), interleukin-6 (IL-6) and interferon-gamma (IFN-γ) is associated with a spectrum of inflammatory-related diseases including cancer, neurodegenerative disease, atherosclerosis and diabetes (Kremer et al. 1996; Forstermann and Sessa 2012). Several studies have focused on the role of xanthones in *G. mangostana* in modulating inflammatory markers in rat glioma C6 cells, RAW264.5 macrophage and bone marrow mast cells (Nakatani et al. 2002; Tewtrakul et al. 2009; Cho et al. 2014).

The xanthones such as α-, β-, γ-mangostin significantly inhibited nitric oxide (NO) and prostaglandin E2 production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells. These xanthones were shown to exhibit anti-inflammatory activity by inhibition of carrageenan-induced paw oedema in a dose-dependent manner in mice (Chen et al. 2008; Syam et al., 2014) (Chen et al. 2014; Syam et al. 2014). Another study revealed that 1,3,6,7-tetrahydroxy-8-prenylxanthone attenuated inflammatory responses in RAW264.7 macrophage cells and TNF-α mediated inflammation in 3T3-L1 adipocytes by alleviating the activation of MAPKs and nuclear factor kappa B (NF-kB) pathway and promoting the expression of sirtuin 3 (Li et al. 2018). Cho and colleagues showed that mangostenone F inhibited the production of NO, inducible nitric oxide synthase (iNOS), pro-inflammatory cytokines and suppressed NF-kB and MAPK pathways in LPS-stimulated RAW264.7 macrophage cells (Cho et al. 2014). Liu and the team revealed that a dimeric xanthone, garcinoxanthones B, inhibited NO production and suppressed NO synthase expression in RAW264.7 macrophage cells (Liu et al. 2016).

Several studies have demonstrated the potential of mangosteen compounds against inflammatory arthritis. For instance, isogarcinol reduced the mRNA expression of cyclooxygenase-2 (COX-2), the level of nuclear factor of activated T cells (NFAT) and IL-2 expression through inhibition of NF-kB pathway in RAW264.7 macrophage cells and reduced ear oedema in collagen-induced arthritis mice (Fu et al. 2014). A study showed that α-mangostin might have a potential therapeutic value for osteoarthritis by inhibition of IL-1β-induced inflammatory cytokines in rat chondrocytes (Pan et al. 2017). Chan and colleagues revealed that α- mangostin and γ-mangostin alleviated mast cell-mediated allergic inflammatory responses by inhibition of IL-6, prostaglandin D2 (PGD2) and leukotriene C4 (LTC4) production and degranulation in phorbol myristate acetate (PMA) and A23187 induced bone marrow-derived mast cells (Hee-Sung et al. 2012). Taken together, the studies indicated that mangosteen derivatives possess the ability to modulate inflammatory markers in different experimental models.

13.3.3 Anti-influenza Activity

Influenza is an infectious respiratory disease caused by influenza viruses type A, B, C and D. The common symptoms associated with influenza include cough, fever, sore throat, runny nose, muscle pain, headache and fatigue (Krammer et al. 2018). Current treatment options for influenza are vaccines and anti-viral agents. Neuraminidase, a key enzyme involved in viral replication, spread, and pathogenesis, is considered as one of the promising targets for combating influenza (Grienke et al. 2012). Clinically used anti-viral agents, such as zanamivir, peramivir and oseltamivir, are neuraminidase inhibitors. Due to limitations, such as drug availability and drug resistance, there is an urgent need for the identification of next-generation neuraminidase inhibitor.

Twelve xanthones from the fruit hull of *G. mangostana* were screened for their in vitro inhibitory potential against bacteria neuraminidase inhibitory activity. Among the xanthones, smeathxanthone A was identified as the most potent nanomolar inhibitor with an IC_{50} value of 270 nM. Kinetic inhibition study revealed that smeathxanthone A was a competitive inhibitor with a *K*i value of 0.15 μM (Ryu et al. 2010).

13.3.4 Anti-tuberculosis Activity

Tuberculosis (TB) is a major health problem worldwide, particularly in low- and middle-income countries in Asia and Africa. It is one of the top 10 causes of death worldwide, with an estimated 1.3 million deaths in 2017 (https://www.who.int/tb/publications/global_report/en/). TB is an airborne disease caused by *Mycobacterium tuberculosis* that affects the lungs. The common symptoms of TB are severe coughing, fever, and chest pains (Fogel 2015). Rifampicin, pyrazinamide and isoniazid are the first-line drug regimen currently used for the treatment of TB. However,

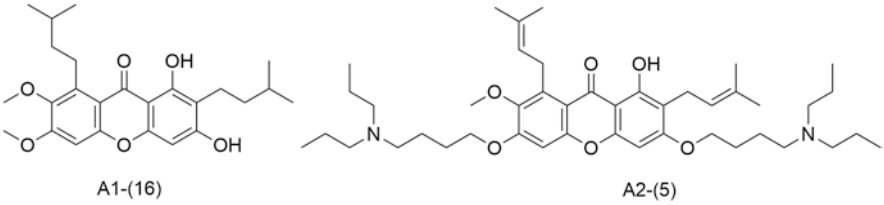

Fig. 13.4 Derivatives of α-mangostin

there is a need to discover and develop a more effective anti-TB drug in view of the *M. tuberculosis* resistance to these current anti-TB drugs. α-mangostin derivatives were evaluated for their antimicrobial potential against *Mycobacterium tuberculosis* H37Ra. Among the derivatives, A-1 (16) (Fig. 13.4) was potently active against *Mycobacterium tuberculosis* with the minimal inhibitory concentration (MIC) value of 0.78 μg/mL (Sudta et al. 2013). A recent study by Koh and colleagues demonstrated that amphiphilic xanthone (A2-(5)) (Fig. 13.4) was active against both *M. bovis* and *M. smegmatis* and able to disrupt the inner microbial membrane and led to ATP depletion. Also, A2-(5) possesses low cytotoxicity, superior metabolic stability and moderate activity against cytochrome p450 enzyme (Koh et al. 2016).

13.3.5 Antidiabetic Activity

Diabetes is a chronic disease associated with high levels of blood glucose in the blood system due to inability or absence of insulin (American Diabetes Association (ADA) 2014). It is implicated with long-term dysfunction and failure of different organs such as eyes, kidneys, nerves, heart, and blood vessels. Several studies have focused on anti-diabetic properties and the mechanisms of action of the extracts and compounds isolated from *G. mangostana*. Loo and Huang (2007) revealed that water fraction of *G. mangostana* containing polyphenols has inhibitory activity with an IC_{50} value of 5.4 μg/mL against alpha-amylase. A study by Hyung and colleagues demonstrated that γ-mangostin (IC_{50} value of 1.5 μM) was potently inhibited α-glucosidase which is an essential enzyme that reduces postprandial hyperglycaemia by suppressing the absorption of glucose (Hyung et al. 2011).

The α-mangostin has proven to enhance insulin production by activating insulin signalling pathway including insulin resistance (IR), pancreatic duodenal homeobox 1 (PDX-1), phosphoinositide 3-kinase (PI3K), Akt and extracellular signal-regulated kinase (ERK) and protected pancreatic beta cells against streptozotocin (STZ)-induced apoptotic damage (Lee et al. 2018). Another study showed that α-mangostin significantly attenuated the high-glucose-induced apoptosis, resulting in up-regulated cleaved caspase-3, Bax, ceramide and enhancement of acid sphingomyelinase activity (Luo and Lei 2017). Preclinical studies demonstrated that ethanol extract was able to reduce postprandial blood glucose levels in STZ-induced hypoglycaemia rats (Hyung et al. 2011). More clinical studies are needed to affirm the potential of α-mangostin as a potential nutraceutical.

13.3.6 Antineurodegenerative Activity

Neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis, prion diseases and frontotemporal dementia, are the primary health problem worldwide. The pathophysiology of neurodegenerative diseases includes memory and cognitive impairments and difficulty to move, speak and breathe. However, despite extensive efforts to unravel mechanisms of disease and discovery of therapeutic agents, neurodegenerative diseases remain incurable and limited treatment options available. Up to date, AD patients rely on cholinesterase inhibitors as a symptomatic treatment since the discovery of disease-modifying drugs to treat Alzheimer's disease remains unmet. The α,γ-mangostin and garcinone C were reported active against acetyl and butyrylcholinesterase enzymes in vitro (Khaw et al. 2014). In vivo study using C57BL/6J mouse model postulated that xanthones rich extract of *G. mangostana* significantly attenuated cognitive impairment, increased brain-derived neurotrophic factor (BDNF) level and decreased p-tau in old B6 mice. On the other hand, the extract was proven to be neuroprotective, anti-oxidative, and anti-inflammatory with reduction of the Aβ deposition and p-tau (S202/S262) levels in the hippocampus of triple transgenic Alzheimer's (3 × Tg-AD) mice (Huang et al. 2014).

Mounting evidence suggested that generation of the neurotoxic Aβ peptide from sequential amyloid precursor protein (APP, a transmembrane protein for neuronal development, neurite outgrowth, and axonal transport) is related to the development of AD (O'Brien and Wong 2011). Aβ peptide, which consists of 38–43 amino acid peptide, was formed after sequential cleavages of APP by β-secretase (BACE 1) and γ-secretase (Chow et al. 2010). Recent findings suggested that both α- and β-mangostin inhibited β-secretase and γ-secretase activity in vitro (Zhao et al. 2017; Lee et al. 2019). Furthermore, α-mangostin was able to attenuate neurotoxicity induced by aβ oligomers. It is shown to inhibit and dissociate aβ aggregation in primary rat cortical neurons (Wang et al. 2012a, b). In a passive avoidance test, γ-mangostin at a dose up to 30 mg/kg significantly improved scopolamine-induced memory impairment in mice (Lee et al. 2019).

Microglia plays an essential role in the progression of neurodegenerative diseases by damaging, injuring and killing neurons (Hickman et al. 2018). A study by Hu and colleagues revealed that α-mangostin at nanomolar concentration attenuated the levels of pro-inflammatory cytokines and NO and reduced ROS production in α-synuclein-stimulated primary microglia cells (Hu et al. 2016). Nava Catorce and colleagues reported that α-mangostin attenuated the levels of IL-6, COX-2 and 18 kDa translocator protein (TSPO) in a peripheral LPS-induced neuroinflammation animal model. Collectively, α- and γ-mangostins are promising candidates to be explored as a therapeutic agent for AD.

13.4 Conclusions and Future Prospects

Over the last 20 years, there has been a significant increase in the research to unveil the potential of xanthones and their semi-synthetic derivatives as an armamentarium against chronic diseases. The α- and γ-mangostins were reported to be effective

against cancer, neurodegenerative diseases and diabetes. Meanwhile, smeathxanthone A and A2 might shed light to fight against influenza and tuberculosis. What makes these xanthones interesting is that it is readily available and possesses minimal toxicity. Although enormous effort has been made to understand the mechanisms of action underlying the pharmacological activities, the clinical translation of the mangosteen compounds is still not possible due to their high hydrophobicity. Low aqueous solubility of xanthones has hindered the absorption, and thus led to poor bioavailability and pharmacokinetic profile. Therefore, formulation and structure modification of xanthone(s) are required to improve the bioavailability of these compounds. Taken together, we believe that there is an enormous potential in the development of the xanthone(s) as a therapeutic agent to address the unmet needs of humankind.

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Capsaicin and Its Potential Anticancer Mechanisms of Action

14

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Contents

Abstract

The human diet and its nutritional content may benefit in preventing several health issues. The enrichment of natural compounds in foodstuffs, having health supporting qualities is the present-day attention of by dieticians to explore a healthy food. *Capsicum* spp., are amongst the enormously consumed fruits

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worldwide, due to their bioactive compounds, capsaicinoids. They are used as nutrition additives and possess pharmacological properties. Hence, hot pepper fruits are widely screened for their pharmaceutical uses in human beings. Capsaicin is the major and commonly occurring capsaicinoids in chilli peppers, and it imparts hot pungent flavour to chillies. It possesses several important biological functions, especially analgesic properties. Capsaicin functions as a neuropeptide-releasing agent, and selective for sensory peripheral neurons. The compound is soluble in fats and readily engrossed through the skin. When used externally, capsaicin controls peripheral nerve ache. Numerous studies have focused on phytochemicals in human diet with anti-tumourigenic or anti-mutagenic phytochemical properties. Several studies performed in animals and cell lines showed the initiation of vigorous apoptosis by capsaicin. Also, it induces cell cycle arrest, reactive oxygen species production and disrupts the loss of mitochondrial membrane integrity, thereby activating caspases activities to promote apoptosis. Further, capsaicin can induce apoptosis in cancer cell lines through increasing the expression of p53 and c-myc genes. In this chapter, scientific evidences describing the potent anticancer efficacy and the mechanisms of action of capsaicin against various types of human cancers are discussed in detail.

Keywords
Cancer · Cytotoxicity · Inhibition · Bioactive compound · Therapeutics · Medicines

14.1 Introduction

Hot chilli pepper, belonging to the family *Solanaceae* and genus *Capsicum* is one amongst the important vegetable and spice crops that are heavily consumed worldwide (Aggarwal et al. 2008). The chief pungent nature present in fruits of capsicum is believed to be mainly because of capsaicin. Capsaicinoid family principally comprises capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homohydrocapsaicin, homodihydrocapsaicin and nonivamide. Currently, there are about 22 naturally occurring capsaicinoid compounds. Each produces a different heat sensation effect in the mouth (Rollyson et al. 2014). The content of capsaicin present in both red and green peppers ranges from 0.1 to 1% (González-Zamora et al. 2015). Substance P is the compound, which depletes the neurotransmitters responsible for stimulating painful nerve impulses. Capsaicin acts as a neuropeptide discharging mediator selective for exterior neurons. When used externally, capsaicin supports to control peripheral nerve ache. Hence, it is used in experimentations for manipulating substance P and tachykinins. Such a characteristic phenomenon allows this compound to be utilized for studying the mechanisms of pain, and also to treat numerous disease conditions involving painful peripheral states (Derry et al. 2017). Capsaicin

has the efficacy of binding with the TRPV (transient receptor potential vanilloid) ion-channel receptors, and it also plays a vital role as an agonist of this ion-channel receptor (Zheng 2013). However, various theories and hypotheses have been put forth to elucidate the biological actions of capsaicin like its anti-neoplastic and cardioprotective properties independent of the TRPV1 receptor (Jara-Oseguera et al. 2008).

Capsaicin is a natural irritant compound, which is unique in character. Stimuli induced capsaicin starts its neuronal excitation at the initial state and later produces new neurons. As a result, neurons stimulated at the initial phase will not produce response, the process which is often referred to as defunctionalization that makes it widely useful in painful disease conditions (Kumar et al. 2013). Numerous studies have focused on phytochemicals in human diet with anti-tumourigenic or anti-mutagenic phytochemicals properties. Capsaicin has been described to induce apoptosis and cell cycle arrest, and decrease the growth of different tumour cells (Clark and Lee 2016). In vivo anticancer action of capsaicin has shown to decrease the cancer cell progression in various animal models. Basith and co-workers have presented that capsaicin exhibits genotoxic and pro-apoptotic actions in cancer cells, but it fails to cause cytotoxicity to normal cells (Basith et al. 2016). Yet, the mechanism through which capsaicin induces apoptosis is unclear. Numerous studies have described that capsaicin induces apoptosis in tumour cells through generating ROS, increasing Ca^{2+} levels, transcriptional factor activation and through loss of mitochondrial membrane potential (Díaz-Laviada and Rodríguez-Henche 2014). The chemistry, biosynthesis and anti-cancerous properties of capsaicin and the mechanism behind its action have been described in many reports. Therapeutic benefits of capsaicin in various cancers have been detailed by several researchers. This chapter compiles all information related to chemistry and anticancer potential of capsaicin in detail. Additionally, molecular mechanisms of anticancer action mediated by capsaicin are discussed.

14.2 About Capsaicin

14.2.1 Chemistry

Compounds classified as capsaicinoids impart the spicy flavour to chilli peppers. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) (69%) is the prime capsaicinoid (Fig. 14.1), followed by others, such as dihydrocapsaicin (21%) and nordihydrocapsaicin (7%) present in chilli peppers. Likewise, chilli fruits also contain capsaicinoids like homodihydrocapsaicin and homocapsaicin in low quantities. The structure of the capsaicin and dihydrocapsaicin varies mainly in the degree of saturation in these capsaicinoids acyl group (Cao et al. 2015). Capsaicin is a lipophilic, crystalline, odourless and colourless alkaloid, having an empirical formula of $C_{18}H_{27}NO_3$ (Reyes-Escogido et al. 2011). It is also known as *N*-(3-methoxy-4-hydroxybenzyl)-8-methylnon-*trans*-6-enamide, 8-methylnon-6-enoyl-4-hydroxy-3-methoxybenzylamide, *trans*-8-methyl-*N*-vanillyl-6-nonenamide,

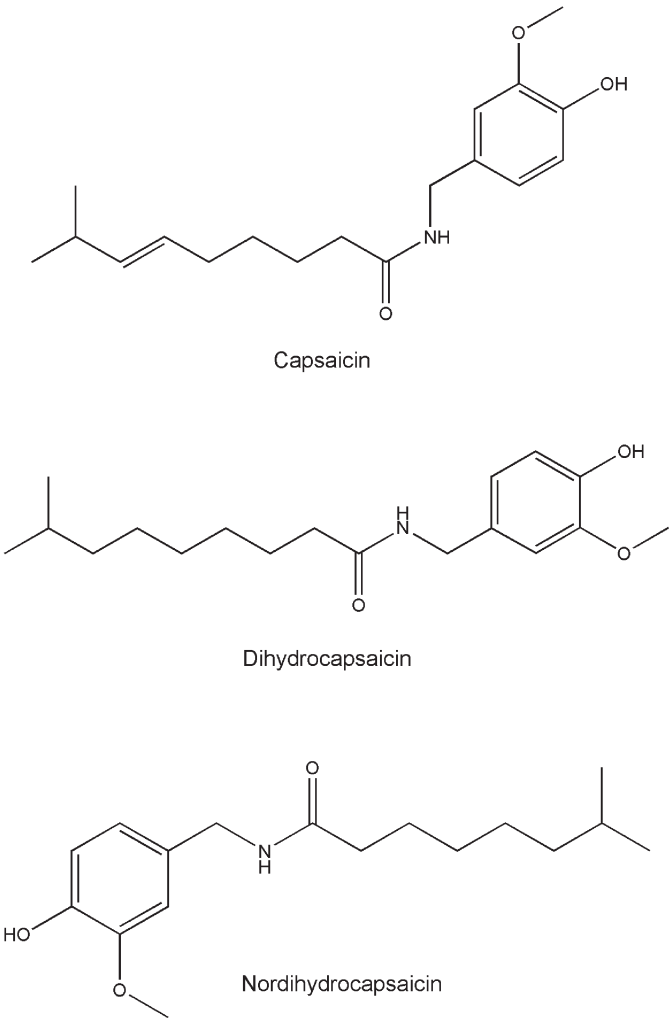

Fig. 14.1 Molecular structures of major capsaicinoids of *Capsicum* spp. fruits

N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-*trans*-6-nonenamide *and* *N*-[(4-hydroxy-3-methoxybenzyl]-8-methyl-*trans*-6-nonenamide. The molecular weight of capsaicin is 305.412 g/mol, and it has a melting point of 65 °C and boiling point of 220 °C. It is soluble in fat, alcohol and oil.

The term capsaicin was coined by Tresh, who first crystallized it in 1876. However, its molecular configuration was validated only in the year 1919 by Nelson and Dawson. Capsaicin exhibits *cis*- and *trans*-isomerism due to the occurrence of double bond that disallows internal alternation. It is mostly found as the *trans*-isomeric form, since the -$CH(CH3)_2$ moiety in the *cis*-form and the elongated chain on the other portion of double bond occur in proximity, leading to a slight repulsion

between them (steric hindrance), which is not noticed in the *trans*-isomer. This added stress makes the *trans*-isomer to have a highly stabilized conformation, when compared to the *cis*-isomer (Arora et al. 2011).

According to the structural activity links for capsaicin agonists, capsaicin molecule has been divided into three parts, i.e., aromatic ring, amide bond and hydrophobic side chain. It has already been established that the substitutes in the third and fourth locations of the aromatic ring are responsible for its effective agonist action, and the 4-OH (phenolic) moiety in capsaicin equivalents (analogues) is of greater significance, because the phenol group can act both as H-bond acceptor/donor, which is pivotal for its agonist action (Katritzky et al. 2003; Reyes-Escogido et al. 2011).

More than 20 capsaicinoids are known so far, and they are all amides produced by the condensation of fatty acids and vanillylamine. Naturally occurring capsaicinoids differ from each other in the number of terminal carbon chain length or the existence/lack of unsaturation (Fig. 14.1). They are biosynthesized in the chilli fruits placenta by the enzymatic reactions mediating in the combining diverse-sized fatty acid chains and vanillylamine. The main enzyme involved is the fatty acyl synthase. The enzyme capsaicin synthase (CS) is responsible for this condensation, which acts on specific fatty acid chain length with the help of cofactors (ATP, Mg^{2+} and coenzyme A). The capsaicinoid structure differs from one another due to the nature of lateral chain length, ranging between 9 and 11 carbons, that varies in the double bond numbers found in different sites lateral to the chain (Reyes-Escogido et al. 2011).

14.2.2 Biosynthesis

Overviewing the agricultural and commercial significance of capsaicin, limited reports are available in relation to the genetics of its biosynthesis mechanism. It is well established that the different genotypes of chilli display extensive difference in the accumulation of capsaicin in retort to genomic, developmental and ecological factors (Rahman et al. 2012). The genetic analysis with regard to capsaicin accumulation was initiated with molecular mapping, which exposed the occurrence of a quantifiable trait locus, known as 'cap' that can be attributed for the enhancement in pungency level. Majority of the genes that were isolated associated with capsaicinoids are concerned with their biosynthesis.

Capsaicin biosynthesis takes place by two paths, namely phenylpropanoid pathway and branched chain fatty acid pathway. The phenylpropanoid pathway decides the structure of phenolic moiety, and metabolism of fatty acid that establishes the fatty acids present in the molecules (Fig. 14.2). Capsaicin's vanillylamine moiety is a derivative of phenylalanine, which is generated through shikimate/arogenate route, while the fatty acid moiety is generated from amino acids (either leucine or valine) (Kehie et al. 2014). It is proposed that the vanillylamine moiety is produced though the phenylpropanoid trail. The vanillylamine moiety in phenylpropanoid descent has been observed in *Capsicum* species, in which the accumulation of

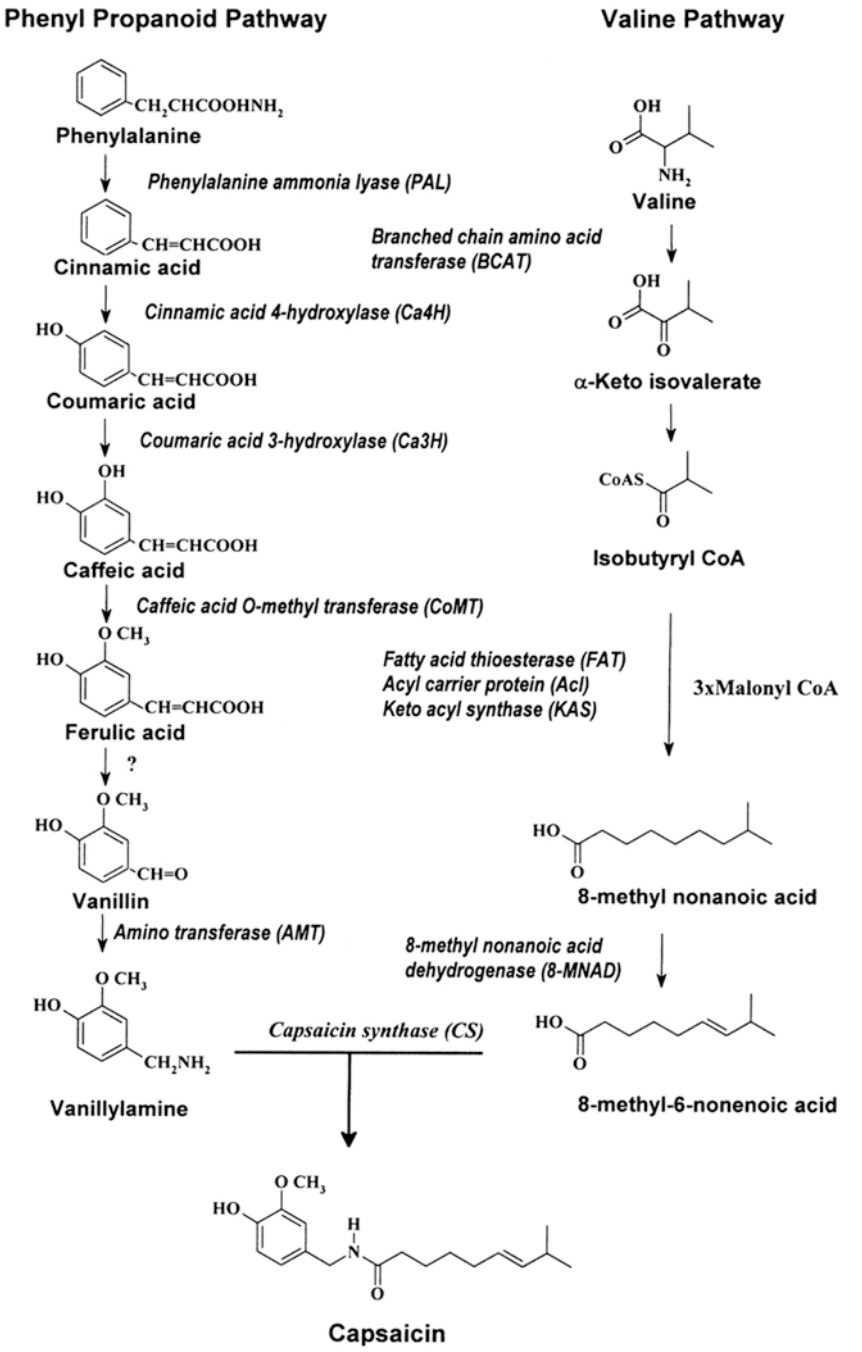

Fig. 14.2 Capsaicin biosynthetic pathway. *PAL* phenylalanine ammonia lyase, *Ca4H* cinnamic acid 4 hydroxylase, *Ca3H* coumaric acid 3 hydroxylase, *CoMT* caffeic acid *O* methyltransferase, *pAMT* putative amino transferase, *CS* capsaicin synthase, *KAS* keto acyl synthase (Prasad et al. 2006). Copyright (2006) National Academy of Sciences, U.S.A.

transcripts has been testified for several enzyme-coding genes, such as PAL (phenylalanine ammonia lyase), Ca4H (cinnamic acid-4-hydroxylase) and CoMT (caffeic acid-O-methyltransferase) that are involved in the phenylpropanoid pathway (Curry et al. 1999). The outcomes of studies by Leete and Louden (1968) and Bennett and Kirby (1968) have confirmed that the branched fatty acid moiety is a derivative of leucine or valine. Findings of Aluru et al. (2003) have demonstrated fatty acid synthase genes (*Kas*, *Acp* and *Fat*) coding for the enzymes that are involved in the metabolism of fatty acids in capsicum fruits. To date, the most intangible opinion in the pathway is the function of CS involved in catalysing the condensation reaction of chain fatty acids and vanillylamine (Kehie et al. 2015).

The concentration of capsaicin exhibits a gradual increment throughout fruit development attaining highest levels between 40 and 50 days, following which, it inclines to break down into minor by-products because of the action of peroxidase enzyme. It has been reported that capsaicinoid levels enhance in exposure to hydric stress because scarcity of water influences the phenylpropanoid pathway (Estrada et al. 1999). In addition, water stress increases the concentration of capsaicin by elevating the enzymatic activity of PAL, Ca4H and CS. Vanillylamine and 8-methylnoneic acid administered as capsaicin precursors had established that vanillylamine occurs at higher levels than 8-methylnoneic acid, and the latter is the rate-limiting substrate in the synthesis of capsaicin (Reyes-Escogido et al. 2011; Kehie et al. 2014).

14.3 About Transient Receptor Potential Vanilloid Subfamily Member 1 (TRPV1)

TRPV1, previously called as the vanilloid receptor also known as the capsaicin receptor, senses heat or warmness in the sensory neurons (Kumar et al. 2013). TRPV1 identified first in rat has a weight of 95 kDa and 830 amino acids in both humans and rats, consisting of six transmembrane domains (Kedei et al. 2001). It is a non-selective receptor which is cationic channel and ligand-operated located in the nociceptive neurons. TRPV1 receptor is broadly distributed in almost all tissues of the body (Cortright and Szallasi 2004; Tominaga and Tominaga 2005) and it couples with a cationic channel, which is permeable to sodium and calcium ions. The receptor is located in endoplasmic reticulum and plasma membrane that controls the intracellular calcium levels (Reyes-escogido et al. 2011). This ion channel can be controlled and activated by substance such as endovanilloids which are produced internally. Various exogenous stimuli brought about by chemical agonists such as capsaicin are highly lipophilic in nature and shares structural similarity with numerous internally produced fatty acids recognized as TRPV1 agonists (Morita et al. 2006).

Burning sensation of capsaicin is brought about by heat-sensitive subunit of TRPV1. Iodo-resiniferatoxin, capsazepine, ruthenium red, A-425619, AMG9810, SB-366791 and SB-705498 are antagonists of TRPV1 (Gonzalez-Reyes et al. 2013). Capsaicin when binds to TRPV1 receptor triggers the release of

neuropeptide substance and stimulates the expression of calcium gene-related peptide (CGRP), thus increasing the calcium levels intracellularly. Due to interaction of capsaicin with sensory neurons, local application of capsaicin in skin promotes analgesic response which is due to desensitizing action of sensory neurons promoted by P substance depletion. This mode of capsaicin action serves as a foundation for numerous studies for developing TRPV1 synthetic ligands. A number of study results show that capsaicin also takes part in release of CGRP, somatostatin and endothelin (Jara-Oseguera et al. 2008; Kumar et al. 2013; Derry et al. 2017).

14.4 Anticancerous Potential of Capsaicin and Its Mode of Action

Several studies performed in animals and cell lines showed the initiation of vigorous apoptosis in various forms of tumour cells by capsaicin. Several additional studies show that capsaicin administered at low concentration exhibit a cancer-chemo preventive and anti-tumour activity (Ramos-torres et al. 2015). From couple of decades research, many literature evidences described the potent anticancer efficacy of capsaicin against various types of human cancers (Surh 2002). Multi-signal targeting ability of capsaicin in various models of cancers induces apoptosis in tumour cells leaving the normal cells uninjured. Such capability of capsaicin makes it available as a beneficial chemotherapeutic agent compared to the other agents which prevent cancer (Surh and Lee 1996).

Capsaicin mediates anti-tumour efficacy not only through interacting with receptors of TRPV1, but via increasing calcium levels intracellularly which in turn initiate apoptosis (Ziglioli et al. 2009). In a study using immortal cell line, capsaicin stimulated apoptosis by reducing the progress and viability of tumour cells, induced cell cycle arrest and significantly disrupted loss of mitochondrial membrane integrity, thereby activating caspase 9 to promote apoptosis (Lin et al. 2013a, b). In addition to this, capsaicin inhibits the activities of electron transport chain complexes I and III in cancer cells, while in the presence of ROS, the inhibition of complexes I and III resulted in cell death (Pramanik et al. 2011).

Studies have cited that capsaicin has the ability to increase the rate of carcinogenesis. In a recent evidence, capsaicin was found to increase the expression of tumour-associated nicotinamide adenine dinucleotide (NADH) oxidase in colon carcinoma cells of humans. Suppression of TRPV1 receptors over a long period increases skin cancer risk (Bode and Dong 2011). Few research have shown that carcinogenic effect of capsaicin is mainly because of by-products produced, which are detoxified by cytochrome P450 (Surh 2002), and many new responsive metabolites were also identified (Kumar et al. 2013).

14.4.1 Prostate Cancer

Prostate cancer is a tumour condition occurring commonly among males. In vitro and in vivo studies of capsaicin in prostate cancer have revealed to obstruct the proliferation of cancer cells (Clark and Lee 2016). High TRPV1 receptor expression in prostate cancer indicates positive association of it during increased malignancy conditions. Thus, it may serve as an exploratory aspect in prostate cancer (Van Haute et al. 2010). Pain management in prostate cancer can be achieved by desensitization of TRPV1 receptors induced by capsaicin (Jara-Oseguera et al. 2008). Using a radio-sensitizing agent, Natalieet et al. specified that capsaicin could have inhibited prostate cancer via inhibiting NF-κB signalling. Other research evidence suggested that capsaicin helps in the secretion of interleukin-6 in PC-3 cells and persuades the activation of ERK, TRPV1, PKC-a and PI3K/Akt (Malagarie-Cazenave et al. 2011). Other evidence presented that capsaicin initiates apoptosis on prostatic cancer cell lines via hindering the stimulation of nuclear factor-kappa B (NF-kB) and tumour necrosis factor-α (TNF-α) (Mori et al. 2006; Malagarie-Cazenave et al. 2011).

In prostate cancer cells, capsaicin induces cell death by increasing the production of ROS, disrupting mitochondrial integrity and activation of caspase 3. Another similar molecule capsazepine was found to inhibit JAK/STAT3 pathway in prostate tumour cells (Huang et al. 2006). Capsaicin promotes apoptosis in PC-3 cells via generation of ROS, and activates numerous molecular signalling cascades which is described using a microarray approach. In association with this, GADD153/CHOP, an endoplasmic reticulum stress-regulated gene expression, was found to be significantly higher and that might have persuaded the antiproliferative effect (Aa et al. 2019). Co-treatment of capsaicin with NAC protected the cells from apoptosis and revoked the inhibition of PI3K/Akt/mTOR partially. Autophagy is the process which is necessary for survival of prostate tumour cells and capsaicin lessens proliferation of cells by blocking through ROS generation in prostate cell line LNCaP (Ramos-torres et al. 2015). Increased expression of IL-6 was found to be stimulated by TNF-α and it is modulated activation of ERK/MAPK signalling (Patowary et al. 2017). Capsaicin mediates cancer cell death through secretion of TNF-α and IL-6 and activates Akt, ERK and PKC-α pathway, demonstrating that capsaicin might serve as an protective anticancer compound in human prostate cancer (Malagarie-Cazenave et al. 2011).

Further, administration of capsaicin orally to animals has been described to lessen the tumour cell development and proliferation, and the expression of NF-κB (Venier et al. 2015). Administration of capsaicin for long duration was revealed to be safe and protective in reducing the process of metastasis in prostate cancer cells (Basith et al. 2016). One of the current miRNA profiling research studies showed that capsaicin controls the androgen receptor activity at the level of protein and mRNA, thereby restoring miR-449a and it increases prostate cancer cell sensitivity through up-regulating the miR-449a expression (Zheng et al. 2015). Capsaicin present in habaneros chilli sauce, when consumed weekly, resulted in significant delay in progression of prostate-specific antigen (PSA) in patients with prostate cancer

(Jankovic et al. 2010) and also it worked effective as a adjunctive treatment in prostate cancer patient (Pramanik and Srivastava 2013). In vivo experiments showed that arvanil (analogue of capsaicin) blocked the growth of prostate cancer cells (DU145, PPC-1, and TSU). Literature evidences regarding capsaicin have reported that it has a profound effect of inhibiting cancer cell growth equally in in vitro and in vivo *models*, through apoptosis induction in both positive and negative receptors of androgen (Mori et al. 2006). Complete study of capsaicin regarding prostate cancer recommends that it would be a capable chemopreventive and anticancer agent for preventing prostate cancer.

14.4.2 Pancreatic Cancer

Pancreatic cancer is the fourth leading deadly form of cancer occurring worldwide (Boreddy and Srivastava 2013). Capsaicin-treated mice with tumours exhibited higher rate of apoptosis together with JNK and activation of other proapoptotic proteins of apoptosis (Zhang et al. 2008). Pancreatic cancer cells such as BxPC-3 and AsPC-1 were treated with capsaicin. Capsaicin displayed reduced activities of oxidative markers such as SOD, glutathione peroxidase, catalase and GSH. (Kodydkova et al. 2013). Researchers described that capsaicin might have brought about apoptosis in animal and cell line models of pancreatic cancer via targeting the redox-sensitive thioredoxin (Trx)/apoptosis signal-regulating kinase 1 (ASK1) complex. Reduced expression of Trx and separation of Trx-ASK1 complex activate ASK1 and other apoptotic proteins subsequently stimulating cell death in pancreatic tumour cells (Pramanik and Srivastava 2012).

Pramanik and Srivastava later assessed the mechanistic action of capsaicin-mediated ROS generation in pancreatic cancer cells. The authors observed that capsaicin decreases activities of complex-I and complex-III in pancreatic cell lines (AsPC-1 and BxPC-3). Moreover, they also found that the levels of antioxidants were lowered in tumour mice treated with capsaicin compared to control (Pramanik and Srivastava 2013). Also an in vivo study using mice demonstrated that apoptosis initiation following treatment with capsaicin was connected with depolarization of mitochondria, ROS production, JNK activation and activation of caspase-3 cascade in pancreatic cells (Zhang et al. 2009). Capsaicin exhibits anticancer activity in K-ras-transformed pancreatic cancer cells via inhibiting NOX cell line models. In animal models, capsaicin showed antitumour activity by promoting the oxidative damage to the xenograft tumour cells (Wang et al. 2015). However, an alternative study assessed the capsaicin efficacy in β-catenin signalling which is associated with β-catenin/TCF-1 complex dissociation and with STAT3 to inhibit growth of cancer cells (Pramanik et al. 2015). When nude mice were treated with capsaicin orally, it showed that the growth of AsPC-1 pancreatic cancer xenografts was reduced markingly and with no side effects (Zhang et al. 2008).

Capsaicin was beneficial in overwhelming growth and encouraging apoptosis through increasing the expression of plasmic reticulum-stress- (ERS) markers and through transcription factor 4 (ATF4) and GADD153 activation to apoptotic

pathway, and also suppressed the growth of pancreatic cancer in vitro and in vivo (Lin et al. 2013a, b). Capsaicin inhibited cerulein-prompted pancreatitis and carcinogenesis. It induced apoptosis, while exhibiting chemoprotective prospective in mice (Benzel and Fendrich 2018). Also, the protective efficacy of capsaicin on pancreatic intraepithelial neoplasia (PanIN) and pancreatitis was proved in LSL-Kras G12D/Pdx1-Cre mice model (Bai et al. 2011). Treatment modalities which are available to treat cancer are associated with increased mortality rate. Therefore, treatment with natural compounds such as capsaicin has fascinated increased attention against various cancers (Grothey et al. 2008).

14.4.3 Stomach Cancer

Stomach cancer, otherwise named as gastric cancer, is a malignant form of cancer that arises from lining of stomach. This form of cancer arises without any symptoms and when the cancer reaches advanced stage, it presents symptoms. Infection of stomach with *Helicobacter pylori* increases stomach cancer risk (Sokolova and Naumann 2017). It is suggested that capsaicin partially inhibits the NF-κB activation induced by *H. pylori* ATCC 43504, and thus might be used as a possible target for development drug against *H. pylori* ATCC 43504 infection (Hyoung et al. 2006). Nowadays, people are least recognized with gastric cancer; however, in the past, it was reported with about 900,000 deaths annually worldwide (Sitarz et al. 2018). Earlier studies suggest that capsaicin has the ability to bring apoptosis in gastric cancer cells of humans and it also acts as a protective anticancer agent against gastric cancer in humans (Park et al. 2014). Capsaicin was also revealed to encourage apoptosis in cisplatin-resistant gastric cancer cells through Aurora-A protein degradation (Huh et al. 2011; Lau et al. 2014).

Substances like vanilloids can bring about the inhibition of NADH (nicotinamide adenine dinucleotide-reduced)-plasma membrane electron transport system and it can promote cell death in transformed cells (Patowary et al. 2017). Studies related to antiulcer activity of capsaicin have reported that it can inhibit acid and mucus secretion, stimulates secretion of alkali and afferent neurons, and thereby helps in treating stomach ulcers. It was also found to decrease the acid secretion, stimulate blood flow and help to heal injury in stomach ulcer patients (Satyanarayana 2006). In vitro models using SNU-1 stomach cancer cell line, when treated with capsaicin, induced apoptosis in cancer cell lines through increasing the expression of p53 and c-myc genes (Ho et al. 2005). Bcl-2 plays a vital role in recent years by acting as a central regulator in mediating the apoptosis, and study performed in human gastric cancer cells also showed that capsaicin has promoted apoptosis via reducing the expression of Bcl-2 (Lo et al. 2005).

Possible chemo preventive efficacy of capsaicin was evidenced in combination of capsaicin and 5-FU. The combination proved to have beneficial effect compared to the individual effect (Cao et al. 2015). Capsaicin-promoted cell death in gastric cancer cells is via activating the pathways which are sensitive to Bcl-2 (Lo et al. 2005). Other studies reported that when capsaicin is administered in high doses, it

was found to be highly toxic and it causes harm both to cancerous and non-cancerous cells (Chow et al. 2007). Thus, this feature of capsaicin needs to be investigated for use of the compound in treating various forms of human cancers. Apoptosis in stomach cancer cells is initiated by PARP cleavage and decreased t-NOX protein expression (Wang et al. 2011). P53 overexpression or other unknown factor might have resulted in inducing apoptosis in SNU-1 cells (Kim et al. 1997). Capsaicin was also found to decrease the expression of p38 MAPK and phosphorylated ERK in human gastric adenocarcinoma cell lines (Park et al. 2014). Capsaicin inhibited IL-8 activation in a time- and concentration-dependent manner.

Contradictorily, Skrzypski et al. suggest that capsaicin inhibits ATPs synthesis in mitochondria to induce cytotoxicity in pancreatic neuroendocrine tumour (NET) cells. Capsaicin-induced generation of ROS also stimulates a secondary effect, independent of capsaicin. N-acetyl-L-cysteine (NAC), an antioxidant, acts together with capsaicin to minimize the production of ROS. Altogether, the results suggest that capsaicin induces cellular toxicity via inhibiting ATP synthesis and disrupting mitochondrial potential in NET cells (Skrzypski et al. 2014).

14.4.4 Breast Cancer

Capsaicin hinders proliferation of cells by cell cycle arrest. During S-phase, the MCF-7 and BT-20 cells were detained following treatment with capsaicin for 72 h (Xie et al. 2016). Genistein, phytoestrogen and capsaicin show synergistic anticancer activity through cyclo-oxygenase 2 and AMPK modulation in breast cancer cells (Clark and Lee 2016). Capsaicin induces cell death in breast cancer stem cells through obstructing the NOTCH signalling pathway (Shim and Song 2015). In a breast cancer model of rat (MDA-MB231), vascular endothelial growth factor (VEGF)-A induced sensory neuronal sensitization in response to capsaicin, a TRPV1 agonist (Austin et al. 2017). Moreover, the antitumour action of RPF151 (a novel capsaicin-like analogue) in animal system was independent of TRPV1, thus suggesting that it has lower efficacy than capsaicin against cancer cells (Ferreira et al. 2015). In MCF-7 cells, capsaicin induced apoptosis via pathways that were independent of caspases (Chou et al. 2009).

Capsaicin has been revealed to prevent the development of ER-positive (T47D, BT-474 and MCF-7) and ER-negative (MDA-MB231 and SKBR-3) breast cancer cells by triggering cell cycle (G0/G1 phase) arrest and cell death. Also, cell cycle arrest in the S-phase and inducing of apoptotic effect in breast cancer cells were observed. They also proposed that apoptosis could have been prompted via the mitochondrial pathway and activation of caspase-7 (Chang et al. 2011). Capsaicin induces cell death in ER-positive and -negative breast tumour cells through modulating EGFR/HER-2 pathway and it displays anticancer effect by nearly 50% in MDA-MB321 breast tumour cells (Thoennissen et al. 2010).

Growth of BT-20 and MCF-7 cancer cells was inhibited to a marked extent by capsaicin via induction of fragmentation in DNA (Chou et al. 2009). In vivo animal systems also presented that capsaicin has potent preventive effect in reducing the

propagation of breast cancer cell lines (Lee et al. 2009). Capsaicin and genistein have profound synergistic efficacy in inhibiting the proliferation of breast cancer cell line through AMPK and cyclooxygenase (COX)-2 modulation (Hwang et al. 2009). Structural activity studies related to capsaicin showed that analogues of capsaicin have higher pharmacological property than the natural dietary capsaicin (Lee et al. 2009).

p38 and ERK regulate autophagy and block cancer cell death mediated by capsaicin in MCF-7 and MDA-MB-231 (MCF-10A) noncancerous and cancerous cell lines (Morre et al. 1995). Chang et al. reported that cell death induction by capsaicin is brought about by mitochondrial pathway independent of caspases or by inhibiting NADH oxidase (Chang et al. 2011; Morre et al. 1995). Capsaicin, along with genistein, has the ability to inhibit mammary carcinoma cells via AMPK and COX-2 modulation (Hwang et al. 2009). Considering all these reports, capsaicin was found to be effective against numerous forms of cancer malignancies.

14.4.5 Colorectal Cancer Cells

Colon cancer occurs mainly in the large intestine, while the cancer in rectum occurs in colon; combining together, they are referred to as colorectal carcinoma. Colorectal cancer is the third foremost cancer-associated death described worldwide. Consuming enormous quantities of vegetables, particularly red chilli pepper has potent antioxidant and antiproliferative action which is positively correlated with reduced risk of colorectal cancer (Morre et al. 1995). NAG-1 gene activated by anti-inflammatory drugs exhibit anti-tumourigenic and pro-apoptotic properties in lung and colorectal cancer cells, respectively (Lee et al. 2010). Kim and co-workers established that when resveratrol is treated together with capsaicin, it was found to impede the colon cancer cells growth and promotes apoptosis in p53-WT cells by elevating the levels of nitric oxide (NO). Elevated nitric oxide production caused by capsaicin significantly resulted in increase in expression of both mitochondrial and death-receptor pathways of apoptosis in cancer cells of colon (Kim et al. 2009). Earlier, other groups have observed that capsaicin with AICAR induced apoptosis through activating AMPK and also augmented expression of acetyl-CoA carboxylase in HT-29 colon cancer cells (Kim et al. 2007). Activation of AMPK by phytochemicals such as capsaicin induces apoptosis in tumour cells (Hwang et al. 2007).

Two separate studies by Kim et al. and Yang et al. have shown that capsaicin generates ROS, disrupts mitochondrial potential through caspase-3 and PARPγ pathway which results in cell death in colon cancer cells (Kim and Moon 2004; Yang et al. 2009). In LoVo and Colo320DM cells, capsaicin caused changes in the cell morphology and induced fragmentation of DNA, diminished the DNA contents and encouraged translocation of phosphatidylserine (hallmarks of apoptosis), which is accompanied by production of ROS and loss of mitochondrial membrane potential, via activating caspase-3 (Yang et al. 2009). Administration of capsaicin intraperitoneally at varying doses significantly reduces the colon cancer cells (Lu et al. 2010).

Colon cancer cells growth can be prevented by capsaicin through reducing β-catenin/TCF-dependent pathways (Clark and Lee 2016). Administration of high doses of capsaicin resulted in significant inhibition of colon cancer cells (Yang et al. 2013). The combined action of capsaicin and 3,3′-diindolylmethane (DIM), displayed cell death and inhibited proliferation through targeting NF-κB and p53, which activates the downstream transcription factors, resulting in apoptosis and proliferation inhibition in colorectal cancer cells (Yang et al. 2013).

14.4.6 Lung Cancer

Lung cancer ranks as the second leading cancer worldwide among men and women while it ranks first in the cause of mortality. Smoking is the foremost reason for developing lung cancer and it has become a major health problem worldwide. There are two major types: the most common is non-small-cell lung cancer (NSCLC), comprising 80%, and small-cell lung cancer, which spreads more quickly (Bray et al. 2018). Lung cancer treatment is highly resistant to various treatment modalities. NSCLC at the advanced stages is the most common type of lung cancer occurring at present. Vascular endothelial growth factor (VEGF) is found to be tremendously expressed in the tumour cells which stimulates initiation and progression of non-small-cell lung cancer (Chakraborty et al. 2014a, b). Tobacco smoke contains aromatic hydrocarbons which are polycyclic in nature such as benzo(a) pyrene [B(a)P], that play an important role in lung cancer (Hecht 1999). Swiss Albino mice treated with capsaicin have been found to promote apoptosis in lung cancer cells which is induced by benzo(a)pyrene (Anandakumar et al. 2009a, b, 2015). Supplementation of capsaicin was capable of minimizing the oxidative changes brought about by B(a)P and thus preserves the stability of lysosomes (Anandakumar et al. 2009a, b). Lung carcinoma-induced mice treated with capsaicin at 10 mg/kg improved the levels of phase I and phase II enzymes and tumour was also reduced to a marked extent (Bley et al. 2012).

Small-cell lung cancer (which represents only 13%) is a tumour considered by early distribution and aggressive metastasis of cancer cells, with less than 5% having a survival rate of 5 years. Hence, identification of compounds to decrease the rate of small-cell lung cancer might be hopefully of novel therapy (Pinchot et al. 2008). In one of the recent reports, TRPV6 receptor and downstream of calpain pathway are the main targets of capsaicin to induce cell death in small-cell lung cancer (SCLC) of humans. Capsaicin was studied for its antiproliferative efficacy against SCLC. Capsaicin inhibits proliferation of tumour cells in SCLC, which is facilitated by the E2F4 pathway. Agents identical to the action of capsaicin, which target E2F4 pathway, might be beneficial in treatment against SCLC (Brown et al. 2010). Capsaicin-treated lung epithelial cells showed substantial reduction in cancer cell growth at varying time intervals, without causing harm to normal cells. In human lung cancer cell line A549, activation of p53 is due to silent mating-type information regulation-1 (SIRT1) activation and decrease in the expression of tNOX. Capsaicin was also described to induce autophagy via stimulating the

activity of SIRT1 deacetylase and it also causes reduced p53 acetylation (Lee et al. 2015). The pro-apoptotic activity of capsaicin was arbitrated by the calcium-calpain dependent pathways. Small-cell lung cancer (SCLC) cells, when treated with capsaicin, prompted improved activity of calpain 1 and 2 up to three-fold in comparison to untreated SCLC cells (Lau et al. 2014).

In non-small-cell lung cancer, p53-SMAR1 activation inhibits the expression of downstream VEGF by degrading HIF1a cancer to restrain angiogenesis, and also other targets including pro-angiogenic factors, COX-2 and PGE2 in the signalling pathways when treated with capsaicin (Chakraborty et al. 2014a, b). Normal wild-type p53 was restored, whereas mutant p53 was degraded by the anticancer efficacy of capsaicin NSCLC of humans (Cho et al. 2017) and it also targets angiogenesis by down-regulating VEGF (Chakraborty et al. 2014a, b). These results suggest that capsaicin can be a possible therapeutic target and cogent therapy of resistant NSCLC for p53-targeting drug discovery. Oxidative damage induced by capsaicin results in activation of p53 and increase in miR-34a expression in NSCLC cells. miR-34a, thus expressed, inhibits the expression of Bcl-2 protein expression, thus retarding the survival of NSCLC cells. Bax activation following p53 results in induction of apoptosis via pathway which is mediated by mitochondrial death receptor (Chakraborty et al. 2014a, b). Thomas and co-workers produced nonivamide (a capsaicin analogue), which was shown to decrease the survival of the BEAS-2B (immortalized human lung epithelial cell line) by overexpressing TRPV1 (Thomas et al. 2011). Nonivamide exhibited potent growth-suppressive activity in TRPV1-OE cells, and this development was mediated by the ROS (Thomas et al. 2012). The apparent potential of capsaicin is responsible for the modulation of extracellular matrix components and proteases causing chemo-modulatory and anti-cancer role in lung carcinogenesis.

14.5 Conclusions

Heat-sensitizing action of capsaicin has attracted attention in various applications related to pharmacology. Application of capsaicin in skin in the form of lotions and creams helps in ailment of musculoskeletal pain. (Anand and Bley 2011; Patowary et al. 2017). Due to this efficacy of capsaicin, various researches are targeting capsaicin for delivering drugs, intradermally. The mechanism through which capsaicin exerts its antiapoptotic action in cancer cells is less understood, and it is believed that it was found to modify the gene expression, which is involved in various stages of cancer development. Capsaicin treated at low doses in rats improved the tolerating ability and the weight gain; food consumption was found to be uniform with that of control rats (Rollyson et al. 2014). Dietary compounds having nutritional value were investigated against several forms of human cancers together with cisplatin, a standard drug used for chemotherapy. The study showed that capsaicin further stimulates the apoptotic effect of standard drug cisplatin in stomach cancer of human and reduces the toxicity induced by cisplatin in various models (Jung et al. 2014).

A short half-life and minimal bioavailability result in the limited use of capsaicin in clinical applications. The synthetic, and also modified capsaicin can be

formulated to decrease the injurious effects of capsaicin and improve its anticancer effect even with low concentration of capsaicin (Clark and Lee 2016; Friedman et al. 2018; Moghadaszadeh-ardebili 2016). Several approaches have been performed to bring the bioavailability of capsaicin. These approaches include forming hydrogels, liposomes encapsulation and iontophoresis, which could bring new hope to cancer patients.

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Biosynthesis, Genetic Regulation and Therapeutic Potential of Capsaicinoids

15

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Abstract

Capsaicinoids are a group of molecules, which impart pungency (hotness) to *Capsicum* fruits. The capsaicinoids are synthesized in the placenta of the *Capsicum* fruits. During the past few decades, there is an increasing interest in elucidation of the biosynthetic pathway of capsaicinoids. The whole genome sequencing of the highly pungent species, *Capsicum chinense* has revealed several important genes that are responsible for the biosynthesis of capsaicin and dihydrocapsaicin, the two major active constituents of the pungency complex, also known as capsaicinoid complex. The capsaicinoids have recently attracted a wide attention, because of its huge pharmacotherapeutic potential in treating several diseases, such as diabetes, cancer, obesity, cardiovascular, respiratory, gastric diseases and urological disorders. Further, capsaicin has been increasingly used as a molecule of choice for pain relief and many other diseases. In this chapter, we have presented briefly the sources and types of capsaicinoids, its biosynthesis, quantification of pungency and the genes responsible for its biosynthesis. Further, we have highlighted the candidate genes that are important for the biosynthesis of capsaicin and can be used for further manipulation experiments. At the end, this chapter focuses on the therapeutic potential of capsaicin in treating several diseases, such as diabetes, cancer, obesity, cardiovascular, respiratory, gastric diseases and urological disorders and its increasing role in pain relief. Finally, we have briefly discussed the mode of action of capsaicin against cancer, cardiovascular diseases, obesity management, pain relief and urological disorders.

Keywords

Capsicum · Capsaicinoids · Pungency · Capsaicin · Functional foods · Specialized metabolites

15.1 Introduction

The species of *Capsicum,* also known as chilli peppers are widely cultivated in different regions of the world, because of their economic importance (Asnin and Park 2015). There are nearly 38 species of the genus *Capsicum*, and only six are domesticated, i.e., *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, *C. pubescens* and *C. assamicum* (Sarpras et al. 2016). Chilli peppers are widely consumed by large population of the world, because of large number of health benefits (Asnin et al.

2013; Sarpras et al. 2016). Their health benefits are attributed to the presence of different compounds, such as capsaicinoids, carotenoids (provitamin A), flavonoids, vitamins (vitamins C and E) and oleoresin (Purkayastha et al. 2012; Sricharoen et al. 2017; Naves et al. 2019). Of the different compounds, capsaicinoids have attracted a considerable attention from the past few decades, because of their diverse roles in treating different diseases. They are genus-specific defensive secondary metabolites that confer variable pungent flavour (hotness) to *Capsicum* fruits primarily to prevent herbivory and secure their fruits and seeds from being attacked by fungi, bacteria and mammals (Sanatombi and Sharma 2008; Tewksbury et al. 2008; Antonious 2018; Arce-Rodríguez and Ochoa-Alejo 2019; Zhu et al 2019). More than 20 analogues of capsaicinoids have been characterized from different species of *Capsicum* (Thiele et al. 2008). However capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (n-DHC), homocapsaicin (h-C) and homodihydrocapsaicin (h-DHC) mainly contribute to the capsaicinoid complex (Lu et al. 2017). Among these, C and DHC account for approximately 80–90% of the total pungency in a chilli fruit (Zewdie and Bosland 2001; Liu et al. 2013; Sarpras et al. 2016). Capsaicinoids are synthesized in the placenta of the fruits making it the most pungent regions of the fruit (Pandhair and Sharma. 2008; Wahyuni et al. 2011; Naves et al. 2019) (Fig. 15.1). Several radioactive and light microscopic studies have also

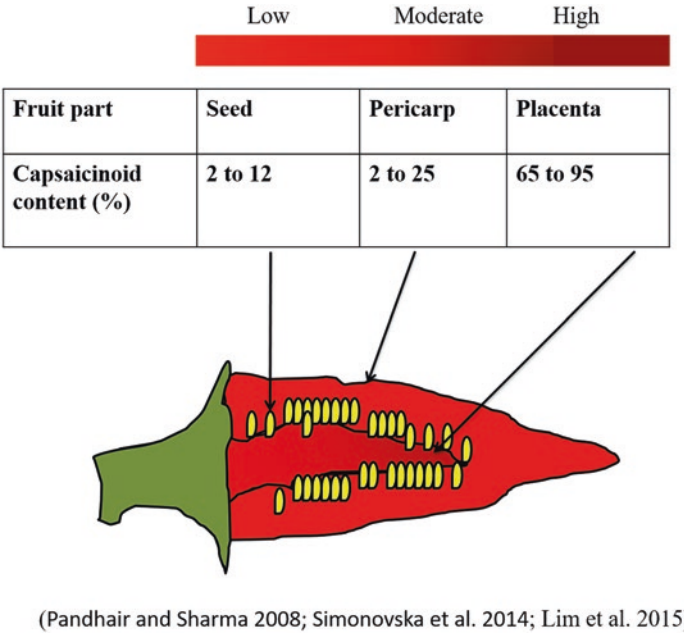

Fig. 15.1 Capsaicinoid content varies in different parts of the fruit; placenta is the most pungent accounting for pungency (65–95%), compared to the pericarp (2–25%) and seed pungency (2–12%) (Modified from Kozukue et al. 2005; Pandhair and Sharma 2008; Simonovska et al. 2014; Lim et al. 2015)

indicated that placenta is the accumulation site of capsaicinoids (Barbero et al. 2016). In recent years, the number of genes have been identified, which are responsible for the biosynthesis of the capsaicinoids by various approaches, such as mutational analysis, gene silencing, transcriptome profiling and expression analysis and whole genome sequencing (Stewart et al. 2005; Kim et al. 2014; Qin et al. 2014; Sarpras et al. 2016; Zhang et al. 2016). The amount of capsaicinoids in the fruits is affected by various factors, such as species of *Capsicum*, its variety, growing conditions, water availability, mineral content, pepper, methods of processing and stage of fruit development (Estrada et al. 2002; Barbero et al. 2016; Ryu et al. 2017). Biotic and abiotic stresses also affect the accumulation of capsaicinoids in the fruits (Tahboub et al. 2008; Gurung et al. 2011; Jeeatid et al. 2017). The total concentration of capsaicinoids in the chillies is expressed in Scoville heat unit (SHU) which was first developed by Wilbur L. Scoville (1912) as an organoleptic test for pungency (Duelund and Mouritsen 2017; Valim et al. 2019). Various techniques such as thin-layer chromatography (TLC), liquid chromatography (LC), supercritical fluid chromatography (SFC), gas chromatography (GC), high-performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GCMS), mass spectrometry (MS), solid phase microextraction gas chromatography-mass spectrometry (SPME/GC-MS) and ^{1}H nuclear magnetic resonance (^{1}H NMR) can be employed for qualitative and quantitative analysis of capsaicinoid content in chilli pepper fruits (Hoffman et al. 1983; Collins et al. 1995; Sato et al. 1999; Reilly et al. 2001a; Barbero et al. 2006; Nazari et al. 2007; Peña-Alvarez et al. 2009; Sarpras et al. 2016; Valim et al. 2019). Besides their use as vegetable and condiment crops, the chilli peppers are an excellent functional food (Mínguez-Mosquera and Hornero-Mendez 1993; Kwon et al. 2007; Dias et al. 2018). Consumption of chilli peppers has proved to have positive effect on human health beyond basic nutrition (Kwon et al. 2007; Meghvansi et al. 2010; Ranilla et al. 2010; Dias et al. 2018). The extract of chilli pepper fruits has been increasingly used for the treatment of deadly diseases such as cancer, type 2 diabetes, cardiovascular diseases, urinary disorders and many other chronic disorders (Johns and Eyzaguirre 2006; Kwon et al. 2007). In this chapter, an attempt is made to give an up-to-date consolidated information on the biosynthesis of capsaicinoids, their quantification and candidate genes that are important for biosynthesis of capsaicinoids. This chapter also pay particular attention to the factors that affect the capsaicinoid content in the fruits and their role as functional foods. We also give a brief outline of the therapeutic potential of capsaicinoids in the treatment of large number of diseases.

15.2 Sources of Capsaicinoids

Capsaicinoids are produced by the members of the genus *Capsicum* in the placenta and pericarp of the fruits. Pungent chillies are commonly known as chilli peppers, whereas non-pungent chillies are known as sweet peppers or bell peppers (Bosland 1996; Luo et al. 2011). Synthesis of capsaicinoids starts in the ovaries prior to pollination; their concentration increases following pollination and reaches maximum

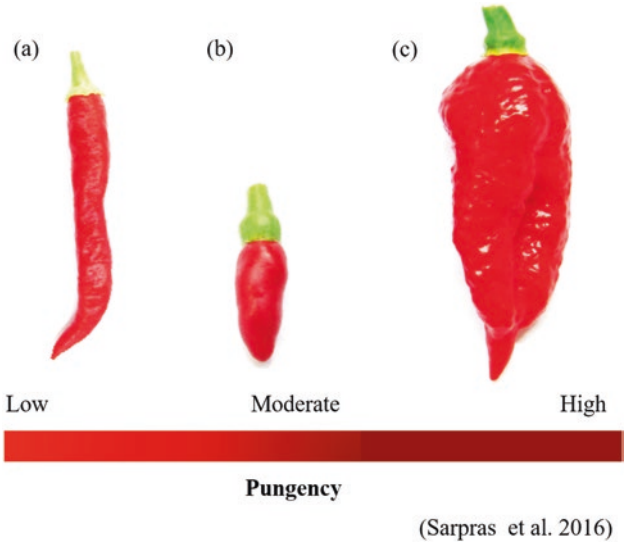

Fig. 15.2 Variation in pungency level (hotness) of different species of *Capsicum*. (**a**) *C. annuum* (less pungent ~<5000 SHU), (**b**) *C. frutescens* (moderately pungent ~0.3–0.5 million SHU) and (**c**) *C. chinense* (highly pungent ~ 0.9–1.0 million SHU; Sarpras et al. 2016)

at the breaker stage (30–45 dpa) (Ananthan et al. 2016; Sarpras et al. 2016; Naves et al. 2019). Different species of *Capsicum* produce varying amount of *Capsaicin* (Fig. 15.2).

15.3 Biosynthesis of Capsaicin (Major Capsaicinoid Responsible for Pungency) Through Phenylpropanoid and Branched-Chain Fatty Acid Pathways

The biosynthesis of capsaicin and dihydrocapsaicin involves convergence of two pathways, i.e., phenylpropanoid and branched-chain fatty acid pathways (Aza-Gonza´lez et al. 2011) (Fig. 15.3). The phenylalanine is produced from chorismate through shikimate pathway in the plastid. After its transport out of the plastid into the cytosol, it is converted into vanillylamine through a series of enzymatic reactions (Mazourek et al. 2009; Baas-Espinola et al. 2016). The other intermediate 8-methyl-6-nonanoyl-CoA is obtained from the pyruvate through a series of steps and enzymatic reactions (Diaz et al. 2004). Conversion of pyruvate to valine first involves the movement of valine into the mitochondria for its catabolism into isobutyryl-CoA and its re-entry back into the plastid. After re-entering plastid, isobutyryl-CoA is elongated to 8-methyl-6-nonenoic acid by the fatty acid synthase (FAT). 8-Methyl-6-nonenoic acid is exported into the cytosol, where it is converted into 8-methyl-6-nonenyl-CoA (Tsurumaki and Sasanuma 2019). The final step involves the condensation of vanillylamine and 8-methyl-6-nonanoyl-CoA which is

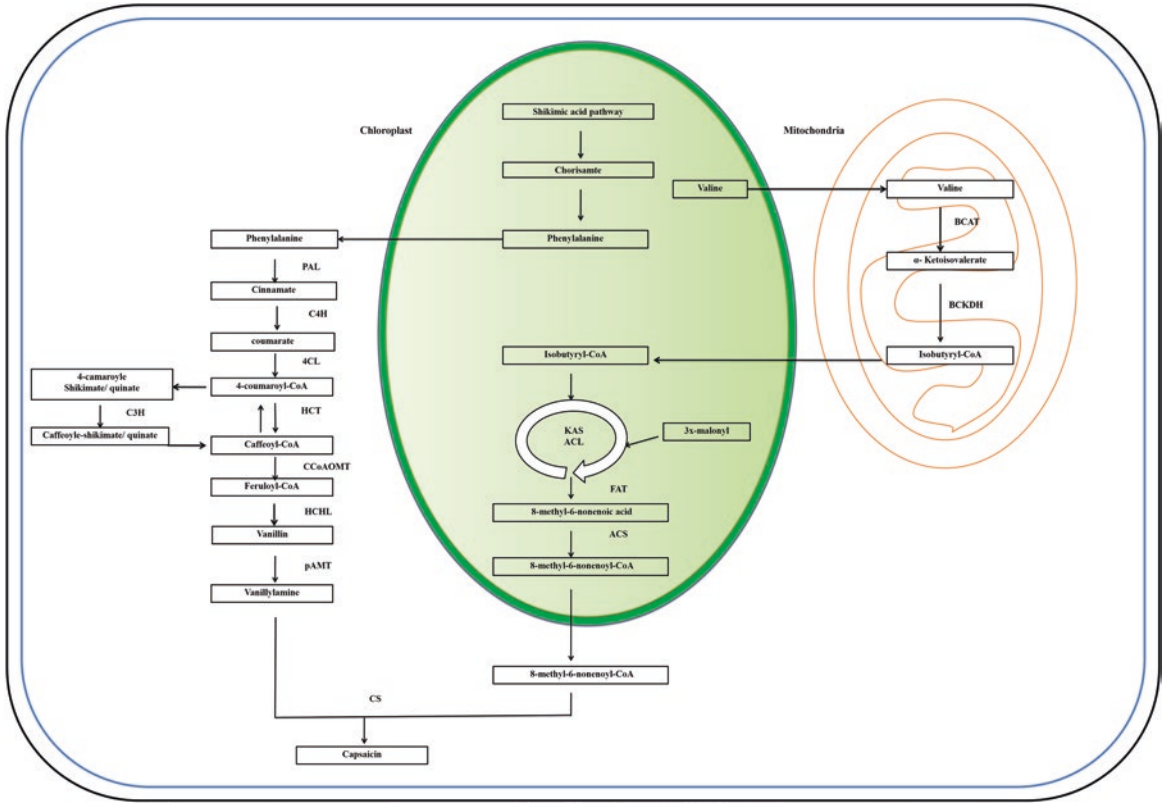

Fig. 15.3 Biosynthesis of capsaicin through phenylpropanoid and branched-chain fatty acid pathways. **Phenylpropanoid pathway:** Chloroplast—phenylalanine is a major precursor for the synthesis of capsaicinoids synthesized from the shikimic acid pathway (Baas-Espinola et al. 2016). Cytosol—phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), coumaroyl shikimate/quinate 3-hydroxylase (C3H), 4-coumaroyl-CoA ligase (4CL), HCT-hydroxycinnamoyl transferase, caffeoyl-CoA 3-omethyl transferase (CCoAOMT), hydroxycinnamoyl-CoA hydratase/lyase (HCHL), putative aminotransferase (PAMT). **Branched-chain fatty acid pathway:** chloroplast—FAT-acyl-ACP thioesterase (FAT), ketoacyl-ACP synthase (Kas), acyl carrier protein (ACL) and acyl-CoA synthetase (ACS). **Mitochondria:** branched-chain amino acid transferase (BCAT), isovalerate dehydrogenase (BCKDH) (Modified from Blum et al. 2003; Aza-González et al. 2011; Kim et al. 2014)

brought by capsaicin or capsaicinoid synthase (CS) (Prasad et al. 2005; Stewart et al. 2005, 2007; Prasad et al. 2006a, b).

15.4 Important Genes Responsible for Capsaicin Biosynthesis

Since two pathways are involved in the synthesis of capsaicin, there are a large number of genes, which are required for the final production of capsaicin. However, through gene expression studies and their correlation with the capsaicinoid content, several key genes have been found to play a very important role in the biosynthesis of capsaicin (Table 15.1). One of the most important genes required for the final step condensation of vanillylamine and 8-methyl-6-noneoyl-CoA is *Pun 1* also known as *CS* or *AT3* (Stewart et al. 2005). Apart from *Pun 1*, several other QTLs have been identified which also contribute to capsaicinoid accumulation in the chilli pepper fruits (Ben-Chaim et al. 2006; Park et al. 2019). Several studies have shown its high correlation with the pungency content in the fruits of *Capsicum* (Stewart et al. 2005, 2007; Reddy et al. 2014; Ogawa et al. 2015). Virus-induced gene silencing of *Pun 1*

Table 15.1 Main genes which are important for biosynthesis of capsaicin

S. No.	Gene name	Location	Role	References
1.	*ACL*	Chloroplast	Conversion of isobutyryl CoA to 8-methyl nonanoic acid in the valine pathway	Diaz et al. (2004), Liu et al. (2012)
2.	*ACS*	Chloroplast	Conversion of 8-methylpentanoic acid to 8-methyl-6-nonenoyl-CoA	Prasad et al. (2005), Kim et al. (2014), Zhang et al. (2016)
3.	*Kas*	Chloroplast	Fatty acid synthesis	Aluru et al. (2003), Kim et al. (2014), Reddy et al. (2014)
4.	*BCAT*	Mitochondria	Conversion of conversion of valine to α-ketoisovalerate	Daiz et al. (2004), Kim et al. (2014)
5.	*FatA*	Chloroplast	Conversion of fat acid to 8-methyl-6-nonenoic acid	Kehei et al. (2013), Kim et al. (2014)
6.	*PAL*	Cytoplasm	Conversion of phenylalanine to cinnamic acid	Liu et al. (2012), Kim et al. (2014)
7.	*C4H*	Cytoplasm	Conversion of cinnamic acid to *p*-coumaric acid	Stewart et al. (2005), Kim et al. (2014)
8.	*COMT*	Cytoplasm	Conversion of caffeoyl-CoA to feruloyl-CoA	Curry et al. (1999), Diaz et al. (2004)
9.	*AMT*	Cytoplasm	Conversion of AMT valine into vanillylamine acid	Prasad et al. (2006a, b); Tsurumaki (2019)
10.	*AT3/ Pun1*	Cytoplasm	Conversion of phenylalanine to cinnamic acid	Stewart et al. (2005), Arce-Rodríguez and Ochoa-Alejo (2015), Tsurumaki (2019)

results in significant reduction of capsaicinoid content (Stewart et al. 2005; Ogawa et al. 2015). *PAL* gene is required for conversion of phenylalanine into cinnamic acid. Some studies have found its role in pungency by expression analysis (Castro-Concha et al. 2016; Zhang et al. 2016). *pAMT* gene is required for the final step in the production of vanillylamine from vanillin. This gene has also been implicated to have an important role in capsaicin production (Curry et al. 1999; Ogawa et al. 2015). Further VIGS and mutational studies indicated its strong involvement in the synthesis of capsaicinoids in the placenta (Abraham-Juarez et al. 2008; Lang et al. 2009; Tanaka et al. 2010). In addition to *PAL*, *C4H* and *COMT* are two more important genes involved in phenylpropanoid pathway (Fig. 15.3, Table 15.1). Very high transcript accumulation of all three genes was reported from the highly pungent habañero (*C. chinense*) as compared to the non-pungent CalWonder (*C. annuum*) species (Curry et al. 1999).

Aluru et al. (2003) initially suggested positive correlation between *KAS, FAT* and *ACL* gene expression and the pungency. Their study showed higher transcript abundance in the placental tissue of the highly pungent *C. chinense* varieties compared with non-pungent *C. annuum* varieties. Similar correlation was also reported by Sarpras et al. (2016). The involvement of KAS was further strengthened through VIGS studies (Abraham-Juarez et al. 2008). VIGS of the *KAS* gene resulted in the reduction in the pungency level of *C. chinense*. FAT gene is involved in branched-chain fatty acid pathway which regulates chain length of fatty acids. Its role in pungency was demonstrated by Aluru et al. (2003). Their study found abundant expression of FAT transcripts in younger fruit tissues suggesting its role in regulation of pungency. The role of ACS in the development of pungency was demonstrated very early using vanillylamine and various forms of methylocatnoyl with capsaicinoid-synthesizing enzyme activity obtained from *C. annuum* var. *annuum* cv. Karayatsubusa (Fujiwake et al. 1980). Zhang et al. (2016) have detected several genes coding for ACS. Their study showed correlation of three ACS genes with the pungency level in pungent *C. frutescens*. *BCAT* gene expression is also found to be consistent with the capsaicinoid in the pungent varieties of *Capsicum* (Stewart et al. 2005; Kim et al. 2014) suggesting their positive role in pungency (hotness). In addition to these 10 important genes, Zhang et al. (2016) have identified 20 more genes by RNA-Seq and DGE that could have an important role in capsaicin biosynthesis. Further whole genome sequencing of the *Capsicum* provides more useful insights into the role of many other genes and other factors such as miRNAs, siRNAs and lncRNAs in the regulation of pungency (Kim et al. 2014; Qin et al. 2014). Recently, studies have also suggested the role of transcription factors in the regulation of genes involved in the pungency (Arce-Rodríguez and Ochoa-Alejo 2017).

15.5 Quantification of Pungency (Hotness) of the Chilli Peppers

The synthesis, accumulation and quantity of capsaicinoids are dependent on the genetic and environmental factors, the developmental stage of the fruit, time of harvesting fruits and season of sowing (Tripodi et al. 2018). The conventional method for quantifying pungency level or capsaicinoid concentration is the organoleptic test, developed by Wilber Scoville (Nagarnaik et al. 2014). This method involves dilution of chilli pepper solution with sugar water until the pungency is detected. Level of pungency is expressed in Scoville heat units (SHU) (Scoville 1912). Conventionally, this method has been in wide usage. Several studies have used this method to quantify pungency level in chilli peppers (Gillete et al. 1984). However, it has been found that this method is objective and prone to errors (Lim et al. 2015). Initially, thin-layer chromatographic method was also used for the determination of capsaicin (Spanyár and Blazovich 1969; Todd et al. 1975; Govindarajan and Govindarajan 1979). Monforte-González et al. (2007) further integrated in situ densitometry with TLC for the quantification of capsaicin.

Most appropriate methods for determination of pungency and quantification of capsaicinoids are based on chemical analysis (Collins et al. 1995; Lim et al. 2015). Researchers are nowadays using recent and advanced methods for quantification of capsaicinoids such as thin-layer chromatography (TLC), HPLC (high-performance liquid chromatography), gas chromatography, (GS), gas chromatography mass spectrometry (GC-MS), liquid chromatography (LC), liquid chromatography mass spectrometry (LC-MS), electrospray ionization (ESI), high-performance liquid chromatography-electrospray ionization-mass spectroscopy (HPLC-ESI-MS/MS) and proton nuclear magnetic resonance (^{1}H NMR) (Deng et al. 2009; Valim et al. 2019).

Several studies have used HPLC method for the quantification of capsaicinoids (Betts 1999; Gibbs and O'Garro 2004; Othman et al. 2011; González-Zamora et al. 2013). It is a common method for determination of capsaicin and dihydrocapsaicin. Some studies have employed advanced tool for this purpose, i.e., HPLC-ESI-MS/MS which is more rapid, simple and sensitive (Garcés-Claver et al. 2006). One advantage of using HPLC-ESI-MS/MS is that it can be used for the simultaneous determination of capsaicin and dihydrocapsaicin (Zhang et al. 2010). Reilly et al. (2001a) have used LC-MS for determination and identification of capsaicinoid analogues in extracts of fresh peppers. Several other researchers have also used LC-MS for capsaicin and its analogues (Liu et al. 2012; Wang et al. 2014; Ma et al. 2016). Liquid chromatography-tandem mass spectroscopy was used by Reilly et al. (2001b) for the analysis of capsaicin, dihydrocapsaicin and nonivamide. Catchpole et al. (2003), Nazari et al. (2007) and Valim et al. (2019) used ^{1}H NMR for the quantification of pungency in chilli peppers. Therefore ^{1}H qNMR (quantitative ^{1}H NMR) can also be used for the quantification of capsaicinoids. It has been found that ^{1}H NMR methods can also be used for rapid and robust quantification of pungency in chilli pepper fruits (Valim et al. 2019; Woodman and Negoescu 2019).

There is an increase in the improvement of methods and machines used for pungency determination nowadays including sensor-based quantification systems. Other methods such as fast GC (Bononi and Tateo 2012), capillary gas chromatography (Thomas et al. 1998) and capillary electrophoresis (Liu et al. 2010) have also been employed. Near-infrared reflectance (NIR) has also been employed for determination of capsaicin using spectrophotometer (Iwamoto et al. 1984). Recently NIR-based real-time measurement system has been developed by Lim et al. (2015) for determination of capsaicin. It is a non-destructive method which scans red pepper powder continuously to reduce the deviation in pungency. A more precise carbon nanotube-based electrochemical sensor has been developed (Kachoosangi et al. 2008). Some sensors have been developed which are used to discriminate between pepper varieties based on the composition of capsaicin and dihydrocapsaicin content (Korel et al. 2002). However, such sensor-based methods are just in the initial stages of use, and research is nowadays also focused on the improvement of these sensors.

15.6 Factors Affecting Capsaicin Content

Pungency, which is an expression of the amount of capsaicinoids, is quantitatively inherited, and its accumulation and synthesis are also affected by the environment (Zewdie and Bosland 2000; Saritnum et al. 2008). The level of pungency is also limited by biotic factors, such as anthracnose disease caused by *Colletotrichum* spp. (Mistry et al. 2008; Ridzuan et al. 2018). In addition to abiotic and biotic factors (Ridzuan et al. 2018), the capsaicinoid quantity is affected by several other factors such as variety/cultivar, conditions of cultivation, level of aging and methods used for processing (Contreras-Padilla and Yahia 1998; Kirschbaum-Titze et al. 2002; Ryu et al. 2017).

Temperature has been found to be an important factor determining the amount of capsaicinoids in chilli fruits. Jeeatid et al. (2017) found increased concentration of capsaicin in *Capsicum chinense* Jacq. at the temperature between 20 and 27°C as compared to high temperature 37°C. González-Zamora et al. (2013) have also found increased accumulation of capsaicinoids in different varieties such as serrano, puya, ancho, guajillo and bell pepper under elevated temperatures. Studies by Murakami et al. (2006) have shown that constant temperatures are important for the increased concentration of capsaicin, whereas fluctuating temperature reduces the quantity of capsaicinoid content. In addition to the temperature, geographical distribution of the chilli peppers also determines the amount of capsaicinoid content (Tewksbury et al. 2006). This study has suggested that populations of *C. chacoense* found at higher elevations show increased pungency levels. Amount of light also affects the quantity of capsaicinoids, and the requirement of light also depends on the species or cultivar under cultivation. Jeeatid et al. (2017) have demonstrated that Bhut Jolokia cultivar produces comparatively more quantity of capsaicinoids under shady conditions as compared to BGH1719 cultivar which shows reduced capsaicinoid content. Díaz-Pérez (2014) has also found that

increasing shade levels up to 35 % leads to increase in pungency. However, further increasing shade level was found to have a negative effect on pungency. Water also plays a very important role in capsaicinoid content in the fruits of chili peppers. *Capsicum annuum* L. var. annuum grown under water-deficient conditions showed increased concentration of capsaicin. The increased concentration of capsaicin also showed positive correlation with its biosynthetic genes, i.e., phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H) and capsaicinoid synthetase (CS; Sung et al. 2005). However, it is not always a rule; in some of the cultivars, the enhanced water stress led to decrease in pungency level (Jeeatid et al. 2018). It is now clear that different genotypes respond differently to water-deficit conditions (Gurung et al. 2012; Phimchan and Techawongstien, 2012; Phimchan et al. 2014). Soil nutrients are also essential for the capsaicinoid content. Among all the nutrients, however, nitrogen and potassium availability is one of the most important factors influencing accumulation of capsaicinoids (Naves et al. 2019). Johnson and Decoteau (1996) and Medina-Lara et al. (2008a, b) have demonstrated the positive effect of nitrogen availability on the pungency of *Capsicum* fruits. Both of these studies have further suggested that increase in potassium did not yield any positive results on pungency. Similar results were obtained later by Monforte-González et al. (2010) and Aldana-Iuit et al. (2015).

15.7 Capsaicinoids as a Constituent of Functional Foods

There is a growing interest in search of foods that have nutraceutical potential and provide health benefits to humans in addition to just nutrition (Sohaimy 2012). Chilli peppers have been in use for centuries in various traditional food systems (Pilcher 2017). Recent scientific studies have shown that chilli peppers are an important source of nutraceuticals and thus comprise an important component of functional foods (Arai 2002). Capsaicinoids and other important metabolites present in the chilli peppers have been considered as an important component of traditional Indian functional foods (Langhans 2018). Besides being nutritional source of several constituents, capsaicinoids have nutraceutical and multiple health benefits such as anti-inflammatory, antiarthritic, anti-obesity, anticancerous, antioxidant effects and improving cardiometabolic activity and increasing the stability of collagen fibres (Materska and Perucka 2005; Maione et al. 2015; Perumal et al. 2015; Clark and Lee 2016; Bogusz et al. 2017; Parvez 2017). The capsaicinoids have been found to be very effective against hyperlipidaemia (Kempaiah and Srinivasan 2006). Several important benefits of capsaicinoids such as anti-inflammatory and pain relief have been summarized in detail by Srinivasan (2007). Capsaicinoids have been found to stimulate thermogenesis that increase fat oxidation, and thus they have been considerably used for weight management (Dulloo 2011; Khan et al. 2013).

15.8 Therapeutic Potential of Capsaicinoids

Since the discovery of capsaicin, there is an increasing evidence in favour of its role in a number of diseases (Pingle et al. 2007; Basith et al. 2016; Fernandes et al. 2016). Because of its multiple therapeutic roles in humans, there is an increasing interest in research on the exploration of different species of *Capsicum* for isolation of capsaicinoids, breeding for higher capsaicinoids, possible use of genetic engineering and gene editing tools in manipulation of pungency. Following subheadings briefly highlight the multiple roles of capsaicinoids in treating different diseases.

15.8.1 Diabetes

It is estimated that 25 % of diabetic patients suffer from peripheral neuropathic pain; the capsaicin creams are one of the several medications recommended for them (Snyder et al. 2016; Derry et al. 2017; Dosenovic et al. 2017). In several studies, researchers have used crude extract components present in the fruits (Tundis et al. 2011; Khan et al. 2014; Sricharoen et al. 2017). Several studies have indicated the role of capsaicin in the management of diabetes and treatment of diabetic peripheral neuropathic pain. Guillot et al. (1996) found a reduction in the glucose levels of the rats treated with capsaicin. Similar results were obtained by Zhang et al. (2017). These experiments also showed a marked increase in insulin levels (Guillot et al. 1996; Zhang et al. 2017). Recent studies have shown that transient receptor potential vanilloid 1 (TRPV1) is activated following treatment with capsaicin (Suri and Szallasi 2008; Zhang et al. 2017). The activation of TRPV1 plays an important role in type 1 (autoimmune) diabetes (Suri and Szallasi 2008).

15.8.2 Cancer

Various studies have indicated the anticarcinogenic effects of capsaicin (Georgescu et al. 2017; Friedman et al. 2018). This has led to an increase in the research for its use against various types of human cancers (Mori et al. 2006; Clark and Lee 2016; Friedman et al. 2018). Studies have shown that capsaicin prevents metastasis of breast cancer cells (Mori et al. 2006). Mori et al. (2006) and Thoennissen et al. (2009) have reported antiproliferative effect of capsaicin on prostate cancer cells. Capsaicin has been found to cause cell cycle arrest at G2/M in human papilloma virus-infected cancer cells (Lin et al. 2012). Several other studies have also demonstrated anticancer properties of capsaicin in in vivo and in vitro cancer cell lines (Huh et al. 2011; Skrzypski et al. 2014; Ko and Moon 2015; Zhao et al. 2016). TRPV1 has been proved as a capsaicin receptor and mediates capsaicin-induced apoptosis by disrupting the mitochondrial and plasma transmembrane potential of tumour cells through the production of reactive oxygen species (ROS) (Macho et al. 2003; Zhang et al. 2008; Pramanik et al. 2011; Santoni et al. 2013; Raphaël et al. 2014; Liu et al. 2016). Capsaicin also alters expression of several important genes

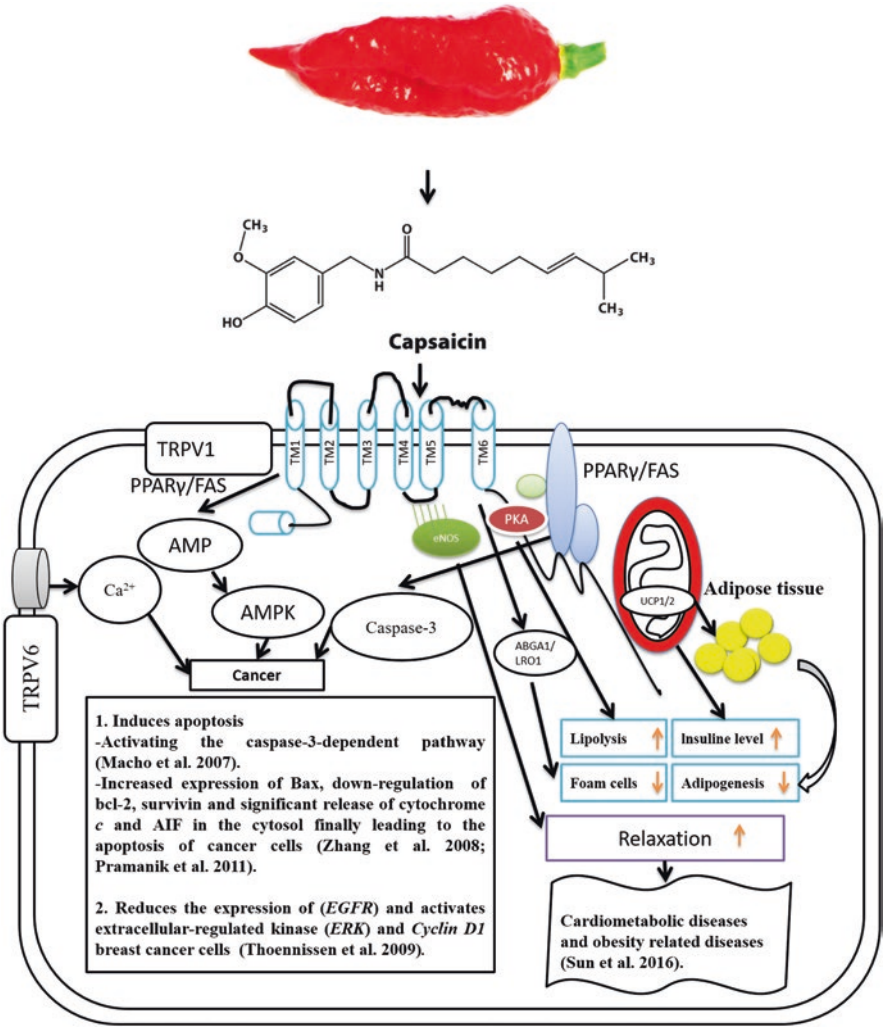

Fig. 15.4 Mode of action of capsaicin against cancer, cardiovascular diseases and obesity management (Modified from Macho et al. 2003; Kim et al. 2007; Zhang et al. 2008; Thoennissen et al. 2009; Yang et al. 2010; Pramanik et al. 2011; Sun et al. 2016a)

that play a key role in cell survival, cell death and angiogenesis (Friedman et al. 2019; Moriguchi et al. 2019). Lu et al. (2010) have reported capsaicin-induced activation of caspase enzymes along with the expression of pro-apoptotic proteins Bcl-2-associated X protein (Bax) and downregulation of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), which helps in cell death of cancer cells. Capsaicin shows anticancer activity by targeting multiple signalling pathways as illustrated in Fig. 15.4 (Zhang et al. 2008; Raphaël et al. 2014; Garufi et al. 2016; Lin et al. 2018).

15.8.3 Pain Relief

Creams made of capsaicin are used to treat peripheral neuropathic pain and diabetic neuropathic pain (Kulkantrakorn et al. 2013; Basith et al. 2016; Yong et al. 2017). European Union (EU) and the USA have approved capsaicin use for postherpetic neuralgia patients (Baranidharan et al. 2013). It has been reported that high concentration of capsaicin (8%) is very effective against neuropathic pain relief (Noto et al. 2009; Baranidharan et al. 2013; Derry et al. 2017). Capsaicin helps in pain relief by desensitization of the local pain nerves through the stimulation of TRPV1 (Caterina and Julius 2001; Knotkova et al. 2008; Peppin and Pappagallo 2013). Several authors have reviewed the exact mechanism of the role of capsaicin in pain relief in depth (Fig. 15.4) (Kress and Zeilhofer 1999; Caterina and Julius 2001; Anand and Bley 2011; Sun et al. 2016a, b). It has now been a well-established fact that capsaicin acts as safe long-lasting analgesia (Knotkova et al. 2008; Remadevi and Szallisi 2008). We have presented a simplified diagram explaining mode of action of capsaicin in pain relief in Fig. 15.5 (for details please refer to Kress and Zeilhofer 1999; Caterina and Julius 2001; Anand and Bley 2011; Peppin and Pappagallo 2013; Sun et al. 2016a, b; Voets et al. 2019).

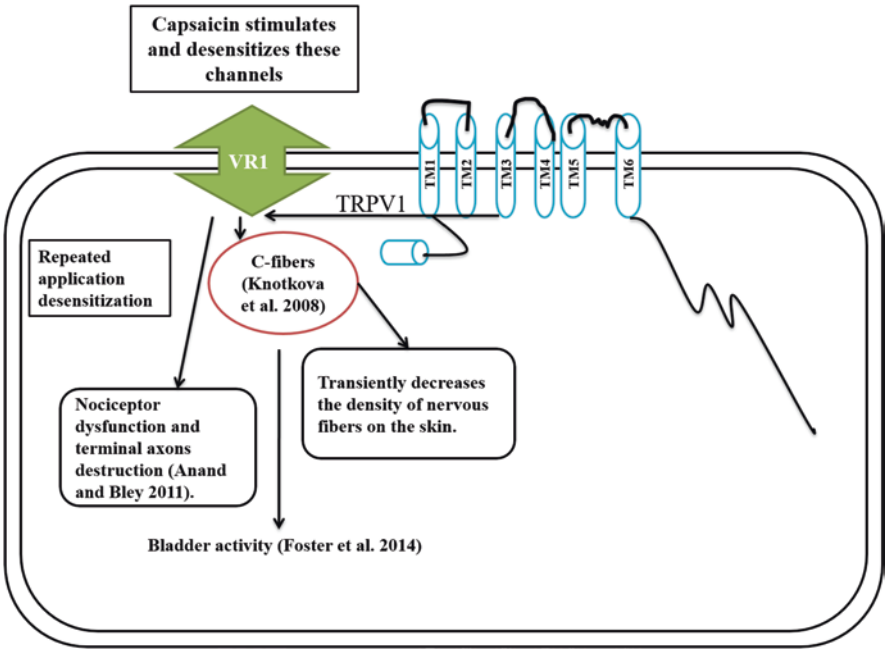

Fig. 15.5 Capsaicin-mediated pathway in pain relief and treatment of urological disorders (Knotkova et al. 2008; Anand and Bley 2011; Foster and Lake 2014)

15.8.4 Management of Obesity

Capsaicinoids have the capacity to act as anti-obesity agent by fuelling the heat production in biological system and preventing the accumulation of fat in body tissues (Ludy et al. 2012; Zheng et al. 2017). Capsaicin hydrolyses the triacylglycerols in adipocytes and mobilizes the lipids and decreases adipose tissue mass (Diepvens et al. 2007; Lee et al. 2010; Lee et al. 2013; Leung 2014). Capsaicinoids are very helpful in the formation of brown adipose tissue mediated via TRPV1 channel-dependent pathway involving the various components illustrated in Fig. 15.4 (Leung 2014; Zheng et al. 2017). Yoneshiro et al. (2012) reported that capsinoids, non-pungent analogues of capsaicinoids, are also help in the brown adipose tissue formation. It has been reported that, following treatment with capsaicin, expression of several genes related to lipid catabolism was increased in mice fed on a high-fat diet as well as obese humans (Diepvens et al. 2007; Leung 2014; Chen et al. 2015). Such studies also suggest a positive role of capsaicinoid-rich diets in the effective management of obesity (Whiting et al. 2014). Several authors have given in-depth details with regard to the mode of action of capsaicin-mediated obesity management (Hsu and Yen 2007; Saad et al. 2017; Lee et al. 2013). Figure 15.4 represents a simplified physiological, metabolic responses downstream of the TRPV 1 receptor following capsaicin binding with it (Whiting et al. 2014; Chen et al. 2015; Tremblay et al. 2015; Voets et al. 2019).

15.8.5 Cardiovascular Diseases

Capsaicin is a good bioactive compound in controlling obesity; it also helps in controlling cardiovascular diseases (Sun et al. 2016a, b). TRPV1 also plays a crucial role in cardiovascular systems and metabolic homeostasis and contributes to controlling cardiometabolic diseases (Ma et al. 2017). It has been reported that capsaicin-induced activation of TRPV1 leads to increased intracellular calcium signalling. This event further exerts many physiological effects (Peng and Li 2010; Sun et al. 2016a, b). Intracellular Ca^{2+} concentration is very important for vascular function and blood pressure (Rylander et al. 2009). Capsaicin-induced activation of TRPV1 leads to anti-hypertension effect (Deng and Li 2005; Sun et al. 2016a, b). Capsaicin also induces an increase in the level of calcitonin gene-related peptide (CGRP) in the plasma and subsequently leads to a decrease in blood pressure (Yang et al. 2010; Sun et al. 2016a, b). It has also been found that capsaicin induces phosphorylation of protein kinase A (PKA) and improves bioavailability of nitric oxide (NO) in endothelial cells (Yang et al. 2010). Several studies have demonstrated an increase in the relaxation of different muscle types (Rocha and Bendhack 2009, Yang et al. 2010; Sun et al. 2013). Adams et al. (2009) demonstrated capsaicin- and dihydrocapsaicin-induced inhibition of blood coagulation and platelet aggregation. Many authors have reviewed the detailed mechanism of action of capsaicin in controlling the cardiovascular diseases (Peng and Li 2010; Sun et al. 2016a, b; Ma et al. 2017; Maiese 2017).

15.8.6 Respiratory Diseases

Another application of capsaicinoids is in managing various allergic, nonallergic airway and chronic respiratory diseases (Gueders et al. 2006; Fattori et al. 2016; Fernandes et al. 2016). Several studies have also shown contradictory role of capsaicin. For example, capsaicin has been claimed to be used against allergic rhinitis (Lacroix et al. 1991; Stjarne et al. 1998), but Seki et al. (2007) and Cheng et al. (2006) have proved that capsaicin does not show therapeutic effect against allergic rhinitis. Instead, Seki et al. (2007) found that capsaicin induces production of interleukin 6 (IL-6) in the airway through the activation of TRPV1 and it causes neurogenic inflammation. Ternesten-Hasséus et al. (2015) have shown that capsaicin decreases cough sensitivity and cough symptoms. Fernandes et al. (2016) have discussed the positive and deleterious effects of capsaicin in cough. Jia et al. (2005) and Jia and Lee (2007) explain in detail the role of capsaicin-mediated TRPV1 activation in respiratory diseases.

15.8.7 Gastric Diseases

There are conflicting results on the use of capsaicin in the treatment of gastric disorders. Makara et al. (1965) have suggested that capsaicin increases ulcer in rat stomach. Szolcsányi and Mozsik (1984) also found similar results. Several other studies have shown contradictory results. A study led by Buiatti et al. (1989) has suggested a protective role of capsaicin-rich diets containing chilli peppers against gastric ulcers. Mhaskar et al. (2013) suggested that consumption of capsaicin-rich diets shows protective effect against *Helicobacter pylori* infection and *H. pylori*-induced gastric ulcer. Satyanarayana (2006) has further suggested positive role of capsaicin in reduction of gastric ulcers. Several authors have discussed the gastroprotective role of capsaicin (Peng and Li 2010; Luo et al. 2011; Srinivasan 2016).

15.8.8 Urological Disorders

Multiple urological disorders affect humans and they seriously affect the quality of life (Groen et al. 2016). Some of urological disorders cause severe pain in the urinary tract and the current drugs do not show satisfactory results (Lazzeri et al. 1996). Intravesical instillation of capsaicin to patients has been proved to control bladder pain and neurological diseases of the bladder (Barbanti et al. 1993; Lazzeri et al. 1996; Cruz et al. 1998). Studies have already shown that TRPV 1 is an important receptor for analgesic drugs. The application of capsaicin activates TRPV1 receptors which leads to desensitization of bladder sensory fibres (Cruz et al. 1998; Foster and Lake 2014). Several authors have given detailed information regarding the role and mode of action of capsaicin in the treatment of urinary disorders (Fig. 15.5) (Foster and Lake 2014; Voets et al. 2019). The mode of action of

capsaicin in amelioration of pain has been reviewed in detail by several authors (Bernstein et al. 1987; Knotkova et al. 2008; Foster and Lake 2014; Smith and Brooks 2014).

15.9 Conclusion and Future Prospects

Many species of *Capsicum* L. show varying amounts of capsaicinoids. Their synthesis and accumulation are determined by various factors, such as biotic and abiotic factors, the species and cultivar grown. Capsaicin and Dihydrocapsaicin are the major components of capsaicinoid complex, determining hotness (pungency) of the fruits. Considering the multiple uses of this wonder molecule, more research is needed for understanding the multiple targets in various cell and tissue types in humans. Capsaicin- and capsaicin-derived drugs have already been in use in recent years. However, scaling up the amount of capsaicin and other capsaicinoids in fruit tissues needs genetic manipulation and genetic engineering approaches. Identification of key genes and their manipulation should be considered for further metabolic improvement of these varieties with increased capsaicin. Consumption of capsaicin-rich diets should also be encouraged as they are also considered to be functional foods with huge pharmaceutical potentials.

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Phytochemicals in the Prevention and Cure of Cancers

16

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Abstract

Most of the early medicines relied on the prescription of specific plants and herbs for medications. This practice is still supported by the present-day research, because of the bioactive phytocomponents present in them. Acting as a defense barrier against several plant pathogens, including bacteria, viruses, and fungi, phytochemicals are also associated with the diminution of lethal diseases in humans, such as hypertension, diabetes, heart disease, etc., and can also effectively diminish the risks of developing certain cancers. Cancer is considered as a neoplastic disease, and despite advances in the modern medicine, it is still the fundamental cause of mortalities in developing as well as developed countries. Specifically, phytochemicals may act as antioxidants and/or nutrient protectors and inhibit the formation of carcinogens (cancer-causing agents) in the body. Furthermore, the potential benefits of phytochemicals as an anticancer agent also include improving the immune system, reducing inflammation, preventing DNA damage, and facilitating DNA repair, thereby slowing down cancer cell growth, regulating hormones, and preventing damaged cells from reproducing. The literature supports the fact that phytochemicals are advantageous, because of their safe, low-toxic, universal availability and their ability to synergize with chemotherapy and radiotherapy. Different studies suggest that regular intake of dietary phytochemicals is allied to low cancer risks. Thus, in this chapter, a summary of the therapeutic perspective of natural phytochemicals and cancer chemoprevention has been presented. Further, different mechanisms of cancer prevention using phytochemicals are discussed.

Keywords

Anticancer · Chemoprevention · Natural compounds · Phytochemicals · Anti-carcinogenic

Abbreviations

ACS	American Cancer Society
Akt	Protein kinase B
AML	Acute myeloid leukemia
AP-1	Activator protein-1
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
bFGF	Basic fibroblast growth factor
BTK	Bruton's tyrosine kinase
CDK	Cyclin-dependent kinases
c-myc	c-Myelocytomatosis
COX-2	Cyclooxygenase-2
CYP1A1	Cytochrome P450 family 1 subfamily A member 1
CYP1B1	Cytochrome P450 family 1 subfamily B member 1

DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
ERK2	Extracellular signal-regulated kinases 2
Fas/CD95	Apoptosis antigen 1 (cluster of differentiation 95)
GSTs	Glutathione S-transferases
HDACs	Histone deacetylases
HepG2	Human liver cancer cell line
HER2/neu	Human epidermal growth factor receptor-2
HIF-1α	Hypoxia-inducible factor-1α
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
i.e.,	That is
ICD-O	International Classification of Diseases for Oncology
JAK2	Janus kinase 2
MAPK	Mitogen-activated protein kinase
MCF-7	Breast cancer cell line
MMP	Matrix metalloproteinase
mTOR	Mammalian target of rapamycin
NCDB	National Cancer Database
NF- κB	Nuclear factor-kappa B
PDGFR	Platelet-derived growth factor receptor
pHDACs	Pan-histone deacetylases
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinases C
PPAR	Peroxisome proliferator-activated receptor
PTEN	Phosphatase and tensin homolog
pVHL	Van Hippel-Lindau tumor suppressor
Raf	Rapidly accelerated fibrosarcoma
Ras	Retrovirus-associated DNA sequences
Rb	Retinoblastoma
Skp2	S-phase kinase-associated protein 2
Src	Sarcoma
STAT3	Signal transducer and activator of transcription
Syk-ZAP-70	Spleen tyrosine kinase-zeta-chain-associated protein kinase 70
UV	Ultraviolet
VEGF	Vascular endothelial factor
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organization
YPEL3	Yippee-like 3 gene

16.1 Introduction to Cancers

16.1.1 Definition and General Types of Cancer

The uncontrollable and abnormal growth of cells by disregarding standard rules of cell division leads to form cancers. Generally, signals for differentiation, division, or death are provided to the cells. But, cancer cells are independently against to these signals, and lead to abandoned growth and proliferation. It can be mortal if proliferation continues and spreads. Various studies articulate that nearly 90% of cancer-allied deaths are attributable to the spread of tumors. Thus, the progression is termed as metastasis (Kumar et al. 2015; Miller et al. 2016). Conferring to different types of cells human body resides, cancer types are distributed into five major categories, i.e., leukemia, carcinoma, lymphoma, myeloma, and sarcoma as well as spinal cord and brain cancers. Their distribution and subtypes are highlighted in Fig. 16.1. The classification chart has been compiled using data available on World Health Organization (WHO), International Classification of Diseases for Oncology (ICD-O) (https://codes.iarc.fr/).

16.1.2 Risk Factors of Cancer

Risk factors of cancer differ from types of cancer as well as the age of cancer patients. According to the National Cancer Database (NCDB) and the American Cancer Society (ACS) (https://www.cancer.org/) database, habitual or lifestyle-related factors, which increases risk of many different types of cancer include smoking, consumption of alcohol, unhealthy food, insufficient exercise, and being overweight. Environmental factors like exposure to different radon, air pollution, and radiations in the course of medical examinations or processes are also accountable in certain adult cancers. However, natural or artificial ultraviolet (UV) is shown to increase risks of skin cancers, including melanoma. These risks usually take several years of impact for cancer risk, so it can be reflected in adult age and not in children or teens, while childhood cancer treated with radiotherapy or chemotherapy can later acquire second cancer, specifically leukemia. Changes in DNA (inherited as well as acquired gene mutations) leads to relax suppressor genes or go for oncogenes that could also trigger cancer, whereas some of the cancers including cervical can be acquired by human papillomavirus (HPV) infections. In addition, risk of Kaposi sarcoma, Hodgkin lymphoma, and a few other cancers can be acquired by human immunodeficiency virus (HIV) infections (https://www.facs.org/quality-programs/cancer/ncdb/about).

16.1.3 Treatments of Cancer

Cancer can be treated by different means unrelated to the stages of cancer and the condition of patients. The traditional therapy includes radiotherapy, chemotherapy, immunotherapy, surgery, as well as targeted therapy (Njuguna et al. 2018). Various

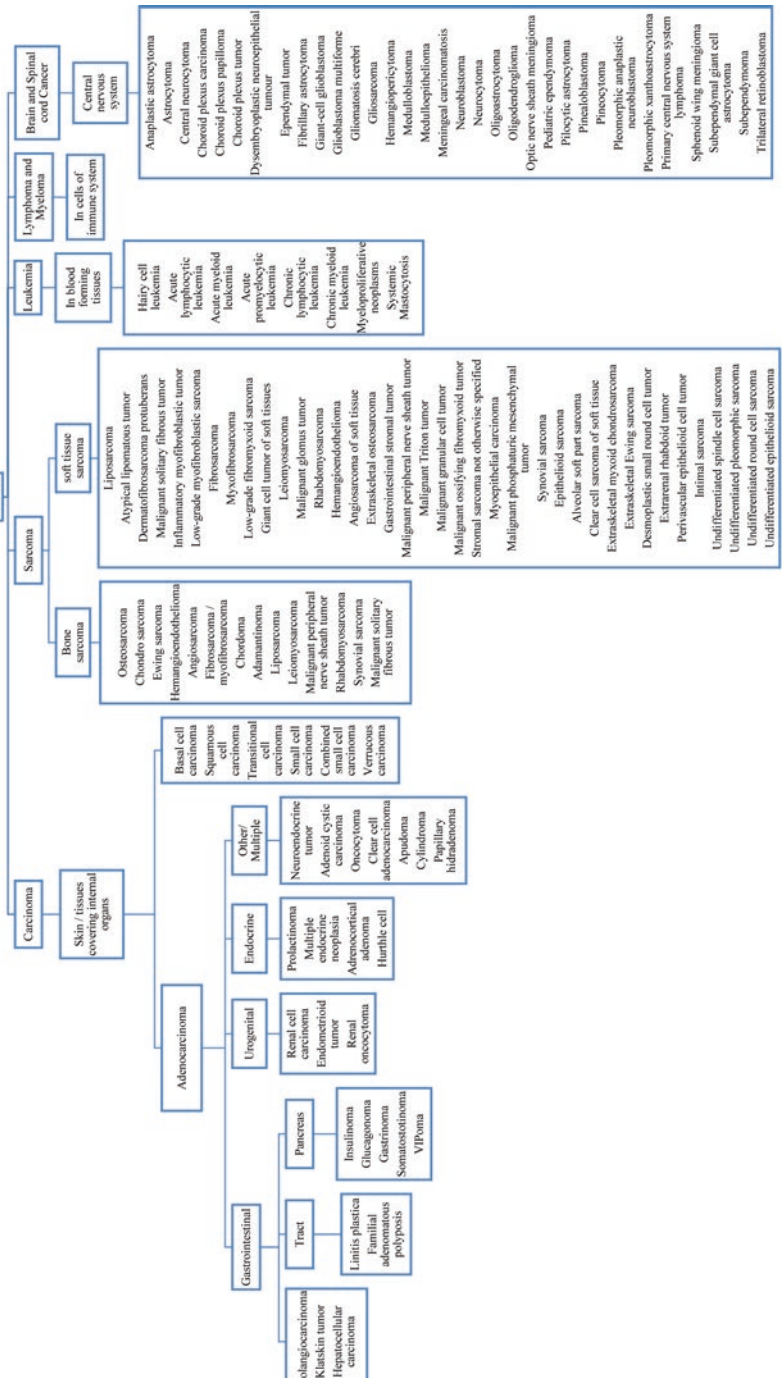

Fig. 16.1 General types and subtypes of cancer depending on different cell functions

new forms of treatments are also considered nowadays which depend on the information about clinical trials. These are deliberated as complementary and alternative therapies comprising photodynamic therapy, stem cell transplant, hyperthermia, donation and transfusion of blood, laser treatment, etc. (Miller et al. 2016; Njuguna et al. 2018).

16.1.4 Side Effects of Anticancer Drugs (Synthetic Drugs)

The most common side effects of anticancer drugs include vomiting, nausea, fatigue, infections (Ayoub et al. 2018), mouth problems, swelling, ostomies, pain, leg cramps (Baden 2015), fever, falling, sleep problems, seizures, eating problems, sweating, weakness, hiccups, changes in thinking or moods, prostheses, low blood counts, peripheral neuropathy, shortness of breath, urine or stool changes, lymphedema, sexual and fertility side effects, emotional side effects (depression, anxiety, distress) (El-Mowafy 2018), skin problems, dehydration (lack of fluids) (Ng et al. 2018), and hair loss (Wallisellen 2019). One of the synthetic anticancer drugs is doxorubicin, which is also effective against acute lymphoblastic leukemia and different breast, ovaries, and hepatocellular carcinomas. It is found that doxorubicin has harmful effects like cardiotoxicity which can lead to heart failure (Chen et al. 2011). Similarly, aspirin and celecoxib used for anticancer treatment caused some side effects like gastrointestinal bleeding, heart attack, and stroke (Cheng et al. 2016), whereas Panhistone deacetylases (pHDACs) inhibitors used as anticancer agent possess several side effects including taste disturbance, diarrhea, fatigue, weight loss, cardiac arrhythmias, bone marrow depression, and electrolyte changes (Prasad and Katiyar 2015). Few chemotherapy drugs, consisting of antimetabolites like methotrexate, hormones, molecular targeting agents, anti-tubulin agents like taxanes, and some DNA-interactive agents like cisplatin and doxorubicin are used for cancer therapy. However, these drugs are escorted with quite a few undesirable side effects like hair loss, bone marrow suppression, neurological problems, cardiotoxicity, and gastrointestinal injuries (Hosseini and Ghorbani 2015; El-Mowafy 2018; Wallisellen 2019). So researchers are hunting for some innovative anticancer agents from natural resources revealing insignificant side effects and enhanced efficacy.

16.2 Phytochemicals: Introduction and Different Mechanisms of Cancer Prevention by Phytochemicals

Phytochemicals are bioactive non-nutrient composites, originated in plants, and are responsible for the pigments, flavor, as well as fragrance of foods (Thomas et al. 2015). Over and above 5000 phytochemicals have been predicted in fruits, grains, and vegetables, while many of them are still unidentified (Hosseini and Ghorbani 2015). Phytochemicals positively affect human health via various mechanisms like signal transduction modulation, epigenetic modifications, regulating antioxidant properties, and modulation of metabolic pathways. Hence, phytochemicals have

comprehensively been studied for their anticancer potential through apoptosis, autophagy, angiogenesis, cellular differentiation, modulation of cancer growth, and its initiation with the aim of reducing side effects like nausea and fatigue (Tabolacci et al. 2019). Many studies have allied that intake of dietary phytochemicals has lowered the incidence of cancer by involving as safeguards in enhancing the repair of DNA, protecting by arming our antioxidant enzymes, and many more (Thomas et al. 2015).

Few plants, as well as phytochemical compounds, responsible for anticancer properties through some of the possible mechanisms are reviewed in this chapter and depicted in Fig. 16.2.

16.2.1 Through Antioxidant Activity

Compounds, inhibiting oxidation reaction that yields free radicals and leads to chain of reactions in order to damage cells are termed as antioxidants. Hence, these antioxidants are essential to maintain integrity of cells as well as homeostasis of the host immune system (Thyagarajan and Sahu 2018). Different antioxidants in foods, including phenols, flavonoids, carotenoids, curcumin, and nobiletin are recognized to be potent for the anti-carcinogenic property. The in vitro studies confirm that supplementing resveratrol- and flavonoid-rich fruits and vegetables in specific proportion lowered the rate of breast, prostate, and renal cancers (Bennett et al. 2012), while the carotenoid mixture helped to suppress hepatoma developed in liver cancer with a high risk as well as help to reduce breast cancer risk. Similarly, nobiletin and curcumin exhibited initiation of antitumor effects (Nishino et al. 2004). In another study, antioxidants like vitamin E and selenium reduced prostate and colon cancer risks. The study revealed that antioxidant vitamins and few phytochemicals help cancer cells to induce apoptosis and prevent angiogenesis as well as the spread of metastasis (Borek 2004). On the other hand, green leafy vegetables, fruits, and dietary quercetin also helped to reduce lung, breast, stomach, colorectal, and prostate cancer (Arsova-Sarafinovska and Dimovski 2013). In a similar way, 6-hydroxy-2,2-dimethyl-3-chrom, rosmarinic acid, 1-O-β-glucopyranosyl-1,4-dihydroxy-2-prenylbenzene, β-sitosterol 3-glucoside, and β-sitosterol isolated from methanol extract of *Gastrocotyle hispida* have shown potent antioxidant and anticancer property (Shahat et al. 2019).

16.2.2 Inhibition of Cellular Mechanisms

16.2.2.1 Cell Proliferation

A condition in which cell number increases, resulting in cell growth and cell division is called as cell proliferation. However, abnormal cell cycle results in accumulation of abnormal cells and its over-proliferation as well. This kind of abnormal and uncontrolled cell growth leads to distinct kinds of cancer (López-Sáez et al. 1998). So, inhibiting cell proliferation of abnormal cells as one of the cellular mechanisms is considered as one of the anticancer assets. It is revealed that polyphenols obtained from green tea also perform as chemopreventive mediators against prostate cancer by

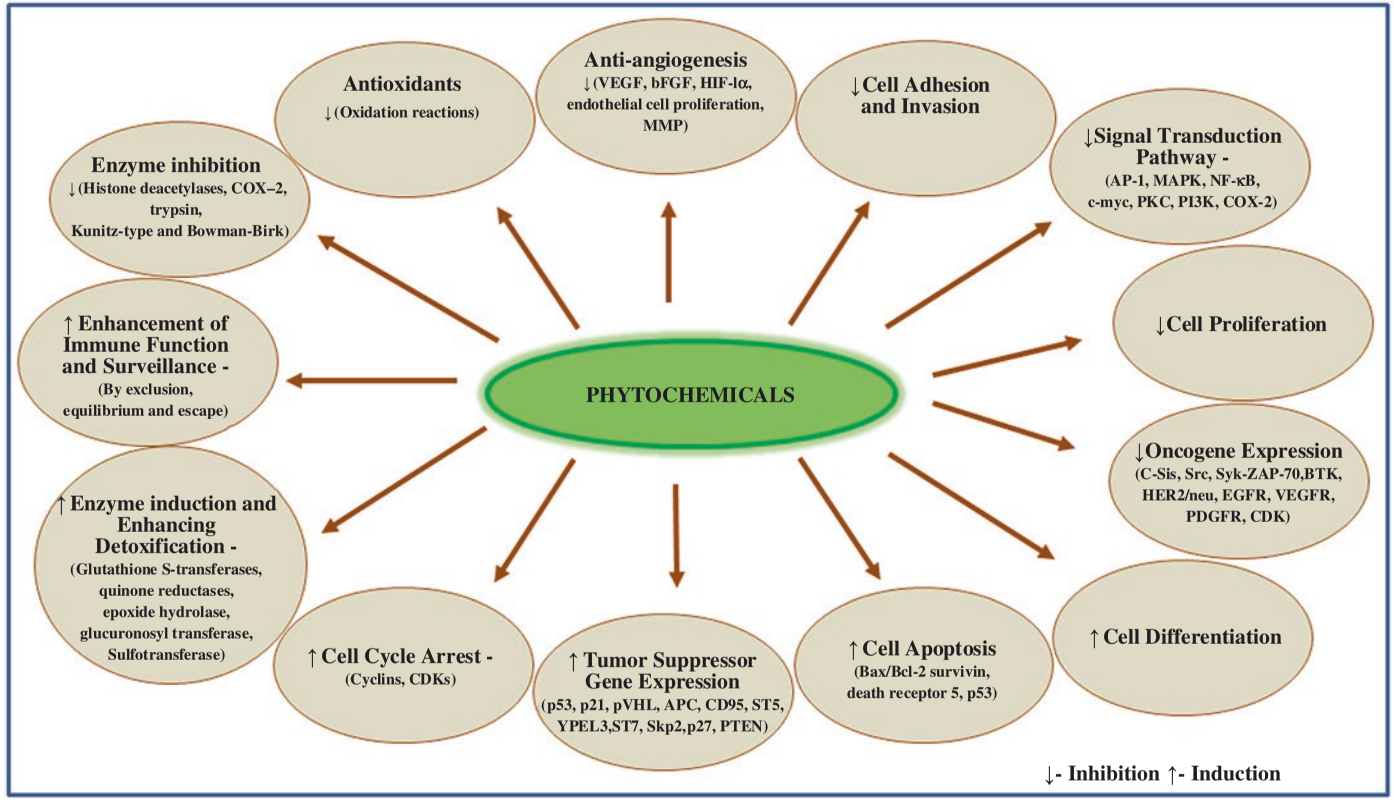

Fig. 16.2 Phytochemicals mediated anticancer potential through some of the possible mechanisms

inhibiting proliferation and inducing apoptosis in cell cultures (Adhami et al. 2004), while methanolic extract of *Oxygonum sinuatum* inhibited proliferation and migration of mammalian endothelial cell (Njuguna et al. 2018). Moreover, anticancer potential of daily diet phytochemical ingredients like curcumin, sulforaphane, lycopene, plus quercetin was studied in colon epithelial as well as cancer cells. Studies here examined mitochondrial activity, DNA synthesis, and lactate dehydrogenase release tests, which are found to inhibit proliferation of colon cancer cell besides not harming the normal colon epithelial cells (Langner et al. 2019), whereas microtubule-targeting agents revealed inhibition potential of the proliferation of cancerous cells. The first group of antimitotic agents was paclitaxel and vinca alkaloids which revealed the capability to inhibit proliferation of cancerous cell (Mukhtar et al. 2014). In a similar way, phytochemicals like vincristine and vinblastine when treated to patients with acute lymphocytic leukemia, testicular cancer, as well as Hodgkin's lymphoma successfully inhibited cell proliferation. Similarly, flavopiridol, fangchinoline, 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone, isorhamnetin, resveratrol, silymarin, and indole-3-carbinol also inhibited proliferation of numerous cancerous cells including colon, breast, kidney, pancreas, prostate, head/neck, endometrial, leukemia, lung, colorectal, bladder, myeloma cancer cell lines, and various other human tumor cells (Bailon-Moscoso et al. 2017).

16.2.2.2 Cell Adhesion and Invasion

A process in which cells form contact with each other or with their substratum through specialized protein complex is called as cell adhesion (Oh et al. 2012), whereas cell invasion is related to cell migration and navigation through extracellular matrix within tissues or to infiltrate neighboring tissue (Pavese et al. 2010). For cancer, adhesion and invasion are the shortest extension and penetration by cancer cells into neighboring tissue for the formation of metastasis. So, inhibiting adhesion and invasion mechanism of cancer cells can be considered as one of the key steps in cancer chemoprevention therapy. Fisetin from strawberries and apples, curcumin from *Curcuma longa* L., and rosmarinic acid found in various medicinal herbs including, thyme, lemon balm, rosemary, sage, oregano, peppermint, as well as culinary spices revealed inhibition potential of cell adhesion, migration, and invasion with variations in their dosages (Wang et al. 2012). Here, curcumin also revealed anticancer property by downregulating the transcription factors, repressing genes responsible for cell adhesion molecules, as well as by decreasing metastasis (Rahmani et al. 2014). Similarly, thymoquinone isolated from seeds of *Nigella sativa* showed anti-metastatic potential by inhibiting cell invasion, cell migration, cell adhesion, and cytoskeletal reorganization when studied on human renal carcinoma cell (Liou et al. 2019), while garlic contains diallyl trisulfide as organosulfur compound reduced matrix metalloproteinase-2 and metalloproteinase-9 (MMP-2 and MMP-9) expressions along with the inhibition of melanoma cell invasion and migration in humans. Here, diallyl trisulfide also disrupted the integrin signal pathways and henceforth inhibited adhesion (Wang et al. 2017). In a similar way, gallic acid, carnosol, caffeic acid, capsaicin, chlorogenic acid, 6-gingerol, 6-shogaol, and their resultant derivatives were proposed to have anti-invasion and anti-metastasis potential (Weng and Yen 2012). In the context of plant extracts,

Porphyra tenera extracts were responsible for inhibition of cell adhesion, migration, and cell invasion in human hepatoma cancer cells. Here, the extracts eventually inhibited gelatinase-A/-B activities (MMP-2/ MMP-9) as well as increased tissue inhibitors of MMP-1/-2 expression by showing anti-metastatic exploit (Do Thi and Hwang 2014), whereas in human lung squamous carcinoma cells, tannin fractions of *Fructus phyllanthi* were responsible for inhibition of cell migration and invasion showing decrease in MMP expressions through regulation of mitogen-activated protein kinases (MAPK) pathway (Zhao et al. 2015). Likewise, root extracts of *Withania somnifera* used in even a small concentration have the potential for inhibition of breast cancer metastasis showing minimal antagonistic properties in rats (Yang et al. 2013).

16.2.2.3 Signal Transduction Pathways

A process in which molecular signals are transmitted from outside to inside the cells is called signal transduction. Generally, these signals affect different functions of cells like cell division as well as cell death. But, cells with permanent changes in signal transduction molecule may form cancer (Liem et al. 2002). So, inhibiting these signals in cancer cells can promote the killing of the cancer cell. Hence, the search for inhibitors of signal transduction pathway could be one of the therapeutic approaches in cancer treatment. The core targets for inhibiting signal transduction pathways are activator protein-1 (AP-1), MAPK, NF-κB, c-myc expression, protein kinases C (PKC), phosphoinositide 3-kinase (PI3K), as well as abnormal cyclooxygenase-2 (COX-2) (Gupta et al. 2017). Ginger consisting of 10-gingerol and 6-gingerol component showed potency to control PI3K/Akt pathway signal transduction which links to anticancer property, whereas sulforaphane found in cruciferous vegetables studied on ovarian cancer cell lines showed an appropriate reduction in PI3K. It also activated phosphorylated levels of Akt and Akt proteins (Suvarna et al. 2017). Similarly, apigenin in endometrial cancer cells has shown particular actions on AP-1 to regulate MAPK pathway, whereas coumarin showed involvement in downregulating pathways like NF-κB, MAPK, as well as Akt. While in combination with paclitaxel, it encouraged a synergistic reduction in the occurrence of the tumor as well as tumor volume when studied in mice (Farrand et al. 2014). However, curcumin, cyanidine, rosmarinic acid, sulforaphane, triterpenoids, resveratrol, and tocopherols were found to inhibit or suppress the activity of COX-2 gene expression in various cancers (Wang et al. 2012; Gupta et al. 2017). Also, in vitro and in vivo trials demonstrated catechol to inhibit c-myc phosphorylation in mice suffering from lung cancer (Lim et al. 2016). Additionally, eugenol found in cloves, bay leaves, nutmeg, cinnamon, and basil showed potential to downregulate the c-myc expression (Ng et al. 2018). Later, in vitro and in vivo studies for anticancer potential carried out using quercetin in combination with cisplatin showed inhibition of the PKC pathway (Purnamasari et al. 2019). Some phytochemicals like benzyl isothiocyanate, genistein, thymoquinone, silibinin, epigallocatechin gallate, parthenolide, naringenin, isoliquiritigenin, ginsenosides, and sulforaphane also suppressed breast carcinoma by several signaling transduction pathways (Younas et al. 2018).

16.2.2.4 Oncogene Expression

Gene responsible to cause cancer is called an oncogene, while a normal gene is a proto-oncogene that may turn to oncogene due to increase in expression or mutation. Generally, these proto-oncogenes code for proteins which regulates cell differentiation and their growth (Croce 2008). So, inhibition of oncogene expression or suppression of proto-oncogene expression using phytochemicals can also help in cancer chemoprevention. Basically, oncogenes are classified in six different categories

1. Cytoplasmic tyrosine kinases including different families (like Src, Syk-ZAP-70, BTK family of tyrosine kinases, and Ab1 gene in chronic myeloid leukemia).
2. Growth factor or mitogens (C-Sis).
3. Receptor tyrosine kinases (including human epidermal growth factor receptor 2 (HER2/neu), epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR)).
4. Cytoplasmic serine/threonine kinases (including Raf kinases and cyclin-dependent kinases (CDK)).
5. Regulatory GTPases (including Ras proteins).
6. Transcription factors (including myc gene, erb A, jun, ets, fos, and myb) (Croce 2008).

Flavonoids inhibiting oncogene expressions were classified based on their activities on cancer cells. Some of them are particularized here. Flavones like luteolin interfered in JNK, p38, and Akt signaling pathways and led to induce programmed cell death along with autophagy in ANA-1 cells. It also showed the potential to inhibit beclin-1 and Bcl-2 together with activation of caspase-3 and -8, while flavonols like galangin induced apoptosis-targeted signals like Akt/PI3K/mTOR resulting in inhibition of cell proliferation in human kidney cancers. However, flavononols like taxifolin inhibited mammary carcinogenesis in LXR-mTOR/Maf1/PTEN and CYP1A1- and CYP1B1-mediated cancers. Similarly, flavans like catechin inhibit programmed cell death ligand 1 to treat lung tumor. Anthocyanidins like delphinidin when studied in human osteosarcoma cell lines showed interference in ERK2/p38MAPK pathway to promote apoptosis and epithelial-mesenchymal transition process (Chirumbolo et al. 2018). A preceding study discussed five phytochemicals, namely, epigallocatechin gallate, kaempferol, genistein, morin, and caffeic acid phenethyl ester for their role in cancer remedy through modulating coding as well as non-coding genes. Some of them are elaborated here. Epigallocatechin gallate showed potency to inhibit expression of neurogenic locus notch homolog protein 2 along with transcription factor HES1 in colorectal cancer and can also target Ras-GTPase-activating protein-binding protein 1 which have chemopreventive effects in lung cancer. Also, kaempferol via downregulating c-myc helps to stimulate ovarian cancer cell apoptosis and can promote the cause of cellular death by reducing Bcl-2 expression and increasing Bax expressions, while genistein helps to target some intermediary signaling pathways like Akt, NFκB, Wnt, and p53 to modulate antitumor activities. Similarly, morin when targeted to Bax, Bcl-2, and cytochrome c in

human leukemia cells showed caspase-dependent apoptosis by means of the intrinsic pathway. Likewise, caffeic acid phenethyl ester targets the NFκB transcription factor to promote programmed cell death in various cell lines (Budisan et al. 2017). Moreover, capsaicin was responsible for the transformation of phenotype in H-ras MCF-10A cells and inhibiting its growth with time-dependent activation of p38 and c- Jun N-terminal kinase-1 along with deactivating ERK-1 and ERK-2, resulting in induction of apoptosis via caspases-3 and fragmentation of DNA. Similarly, rocaglamide as leukemia (Jurkat T, AML) cells treatment showed interference in the intrinsic death pathway by modulating MAPK activities and thereby showing the induction of apoptosis (Kaur et al. 2018). In a similar way, gingerols, emodin, ginsenoside RG3, honokiol, parthenolide, triptolide, wogonin, thymoquinone, resveratrol, andrographolide, quercetin, apigenin, and many more phytochemicals have conferred their ability to inhibit PI3K pathways targeting Akt in several human cancers (Suvarna et al. 2017).

16.2.3 Induction of Cellular Activities

16.2.3.1 Cell Differentiation

A progression in which cells become specialized so as to perform specific functions including neuron, liver cells, or blood cells is referred to as cell differentiation, whereas in cancer, well-defined cancer cells appear more similar to that of normal cells and tend to spread and grow slower than that of undifferentiated cancerous cells. Here, a differentiation term differs for respective types of cancers (Yan and Liu 2016). Hence, drugs inducing differentiation are deliberated as one of the promising lines of attack in cancer treatment. In this regard, all-trans retinoic acid (also called tretinoin), one of the anti-acne medications also used for acute promyelocytic leukemia, was found to induce differentiation in various types of cells and thus effectively used in cancer prevention and treatment (Liu et al. 2015). Similarly, compounds like hydroxyzine, promethazine, levomepromazine, and buclizine were responsible for inhibition of cancer cell growth via direct binding to the translationally controlled tumor protein expression and inducing the cell differentiation of leukemia cells and breast cancer (Seo et al. 2017). In a similar manner, natural phenol resveratrol from peanuts, red grapes skin, and other fruits when studied in human promyelocytic leukemia induced cell differentiation and thus possessed cancer chemopreventive potential (Wang et al. 2012), while a natural isoflavon, i.e., genistein, found in *Genista tinctoria*, soybeans, chickpeas, and other legumes promotes melanoma cell differentiation by stabilizing protein-linked DNA strand breakage as well as by inhibition of angiogenesis (Ng et al. 2018). Moreover, methanolic extract of *Biophytum sensitivum* Dc plant when tested on Ehrlich ascites carcinoma cells and Dalton's lymphoma ascites showed increased count of white blood cells and bone marrow cells along with enhancement of stem cell differentiation (Manisha and Kumar 2018). Additionally, fish oil fats also considered as food phytochemicals were found to increase colon cell differentiation rates (Ferrari 2004).

16.2.3.2 Cell Apoptosis

Cell apoptosis means cellular suicide. It is also mentioned as a process of programmed cell death or self-destruction of cells. Cell necrosis is the premature death of living tissues and cells (Wong 2011). Here, flaws in mechanisms of physiological apoptosis or necrosis contribute to unrestrained cell expansion leading to the progression and development of cancer. So, inducing cell apoptosis or necrosis is an utmost important strategy in cancer therapy. The chief polyphenol catechin called epigallocatechin-o-gallate isolated from green tea extract (*Camellia sinensis*) interfering in cyclic guanosine monophosphate-dependent pathway or acid sphingomyelinase pathway in chronic myeloid leukemia cells played a crucial role to induce cellular death providing anticancer properties (Huang et al. 2015). Hence, epigallocatechin-o-gallate can be useful for the treatment of bladder, cervical, brain, and prostate cancer (Wang et al. 2012). Similarly, the essential oil obtained from *Myrtus communis* L. revealed cytotoxic activity by apoptosis mechanism thereby suggesting it as a natural anticancer compound, while a compound myrtucommulone derived from leaves of *M. communis* revealed increased caspases 3 and 9 activities. It also further released nucleosome in cytochrome c as well as cytosol from mitochondria resulting in DNA fragmentation cleavage along with poly-ADP-ribose polymerases associated with cell apoptosis (Harassi and Ajouaoi 2019). Likewise, curcumin found in turmeric when administered in human lung cancer cells induced apoptosis. Studies also revealed that curcumin helps to induce death in pancreatic adenocarcinoma cells and reduce numerous apoptosis inhibitors (Radhakrishna Pillai et al. 2004; Díaz Osterman et al. 2016). However, a triterpene like-cucurbitane called as 3β,7β-dihydroxy-25-methoxycucurbita-5,23-diene-19-al found in *Momordica charantia* (a wild bitter gourd) helped to induce autophagy and apoptosis in breast cancer cells via peroxisome proliferator-activated receptors (PPAR)-γ (Weng et al. 2013). Phytochemicals of *Allium sativum* clinically tested in colorectal and pancreatic cancer patients increased number and activity of natural killer cells (Hosseini and Ghorbani 2015), whereas saffron a rich carotenoid includes two major components, i.e., crocin and crocetin. According to the in vitro, in vivo and preclinical facts, saffron and its constituents when consumed in daily diet revealed antitumor potential (Bhandari 2015), while saffron isolated from *Crocus sativus* L. exhibited apoptotic and cytotoxic induction in lung cancer cells (A549) (Samarghandian et al. 2013). Two different concentrations (i.e., 0.1% and 1%) of *Thymus vulgaris* L. extract were studied in vivo and in vitro against breast carcinoma. Both doses showed a significant decrease in the mitotic activity index. Moreover, the 4 T1 (breast cancer cell line derived from mammary gland tissue of mice) necrosis/tumor area was reduced to 85% and 84% by treatment with 0.1% and 1% of extract, respectively, as compared to the control rat models. Additionally, the 1% extract reduced the frequency of tumors by 53% in comparison to control (Kubatka et al. 2019). Similarly, anticancer potential of fruit and leaf extracts of *Ficus carica* studied in Huh7it cells revealed the occurrence of necrosis and apoptosis at a high percentage in the presence of leaf extracts (Purnamasari et al. 2019). Also, various mechanisms like apoptosis, cytotoxicity, autophagy, and interference with signaling pathways were observed in the presence of *Piper nigrum* revealing anticancer potential against colon, prostate, cervical, and breast cancer cell lines (Takooree et al. 2019).

16.2.3.3 Tumor Suppressor Gene Expression

Tumor suppressor genes are mentioned as normal genes with the ability to repair DNA, delay cell division, or decide cell death. When the tumor suppressor gene loses the ability to work in a proper way, cells grow in an uncontrolled manner which later forms cancer (Guo et al. 2014). So, inducing expression of tumor suppressor genes may possibly be beneficial in cancer therapy. The first tumor suppressor gene discovered is the retinoblastoma (Rb) gene followed by pVHL, APC, CD95, ST5, YPEL3, ST7, ST14, Skp2, p27, PTEN, and one of the most important ones being the p53 gene (Osborne et al. 2004). Recent reports suggest that phytochemicals induce the expressions of tumor suppressor genes. In this regard, luteolin, a flavonoid, induced Fas/CD95 expression and activated caspase-8 in HLF hepatoma cells. It was also observed that luteolin has the ability to decrease Tyr phosphorylation of STAT3 (which is recognized to act as a negative regulator of Fas/CD95 transcription) and increase the expression of Fas/CD95 (Imran et al. 2019). Curcumin from turmeric has potential to control the progression of tumor cells by inducing tumor suppressor pathways p53 and p21. It also upregulates p16 and numerous other tumor suppressors, JAK2 and STAT3 pathway inhibition, which leads to decrease in smooth muscle actin and migration/invasion capability of breast cancer-associated fibroblasts (Wang et al. 2012; Younas et al. 2018). However, kaempferol from tea, broccoli, and grapefruit interferes in the intrinsic pathway and activates p53 to induce apoptosis in the ovarian cancer cell. Later, kaempferol also proved to induce PTEN expression and Akt inhibition in bladder cancer (Wang et al. 2012; Budisan et al. 2017). Induction of p53, as well as inhibition of STAT3 and NF-κB, was observed due to apigenin in HER2-overexpressing MCF-7 breast carcinoma cells by extrinsic pathway (Younas et al. 2018). In another recent study, it was found that the major component of *Ficus carica* quercetin when individually treated with HepG2 liver cancer cells reported to stabilize p53 and increase Bax/Bcl-2 ratio leading to apoptosis (Purnamasari et al. 2019).

16.2.3.4 Cell Cycle Arrest

A situation when the cell detects any defects or any damage that occurs in DNA, an arrest occurs through several mechanisms to delay or halt the cell cycle. In cancer cells, genetic mutations occur which leads to regulatory malfunctioning and uncontrolled cell proliferation (Rastogi and Mishra 2012). Hence, induction of cell cycle arrest in cancer cells using phytochemicals may be considered as another cancer therapy strategies. In this context, various experimental and preclinical procedures proved that tea flavonoids containing epigallocatechin-3-gallate possess a key role in different cancer treatments via inducing cell cycle arrest (Gödeke et al. 2013). In addition, ethyl acetate extract of *Annona muricata* leaves showed cell cycle arrest in G1 phase associating antiproliferative effect and induced apoptosis through mitochondria-mediated pathway when studied against colon and lung cancer cells individually (Zorofchian Moghadamtousi et al. 2014). Studies based on breast cancer cell lines show that different phytochemicals help to promote cell cycle arrest in different ways. Few of them includes ginsenoside Rg5, a-mangostin, apigenin, isoliquiritigenin, sulforaphane, and curcumin. Here, apigenin has the potential of

inducing cell cycle arrest by suppressing CDK-1, cyclin A, and B that are important for G2 to M phase transition in the cell cycle, while ginsenoside Rg5 helped arrest at G0/G1 phase of the cell cycle by upregulating p21, p53, as well as p15 and down-regulating CDK-4, cyclin E2, and cyclin D1in breast cancer cell lines. Additionally, curcumin along with folic acid enhanced arrest at the G2/M phase of the cell cycle (Younas et al. 2018), while prostate cancer cells treated with caffeic acid phenethyl ester regulated the expression of Skp2, p21, p53, Cip1, as well as p27Kip1 genes leading to induction of cell cycle arrest and inhibition of cell growth (Budisan et al. 2017). Similarly, several mechanisms like activating p53, cell cycle arrests in G0/G1 phase and S phase were triggered by quercetin in leukemia and colorectal carcinoma, respectively. Additionally, G2/M phase arrest was also triggered by quercetin in breast cancer, esophageal cancer, as well as leukemia cells (Purnamasari et al. 2019). Moreover, the aqueous extract of white coca tea to treat human prostate cancer cells revealed cell cycle arrest in G2/M phase when studied in vitro and in vivo, while isoquercetin in addition to silymarin blocked G1 phase of the cell cycle when studied in human liver cells as well as G1/S phase when studied in prostate, ovary, colon, bladder, lung, and breast tumor cells. Similarly, propolis arrested S/G2 phase of cell cycle and inhibited the growth of tumor (Bailon-Moscoso et al. 2017). In a similar manner, sugiol, oridonin, honokiol, gallic acid, and indole-3-carbinol arrest cell cycle in the human breast, prostate, colon, and other different cancerous cell lines (Kaur et al. 2018), while lycopene induced arrest at G0/G1 phase of cell cycle and/or accumulated at S phase in colon cancer cells. Moreover, a recent study shows the synergistic effect of a cocktail containing resveratrol, genistein, C-phycocyanin, indol-3-carbinol, curcumin, and quercetin-induced cell cycle arrest in breast cancer (Langner et al. 2019).

16.2.4 Other Mechanisms

16.2.4.1 Enzyme Induction and Enhancing Detoxification

Generally, enzymes help to accelerate the chemical reaction in the body and participate in many cell functions including cell signaling, growth, and division. Hence, induction of enzymes activity through phytochemicals is one of the trending approaches towards cancer therapy. Therefore, glutathione S-transferases (maintain integrity of cells and protect against DNA damage through catalyzing conjugation of glutathione), quinone reductases (the enzymes present in cells that make certain molecules less toxic), epoxide hydrolase, glucuronosyl transferase (catalyzes broad range of glucuronidation of xenobiotic along with endogenous compounds), and sulfotransferase (enzymes that catalyze sulfonation) are few enzymes involved in phase II metabolism and induced by some phytochemicals or its bioactive compounds which are highlighted below (Dellinger et al. 2012; Abdull Razis et al. 2014; Schnekenburger et al. 2014). Camptothecin can be obtained from numerous plants including *Canzptotheca acirminata* and *Mappia foetida* that have the potential to target topoisomerase I (enzymes involved in DNA supercoils relaxation) to contribute to antitumor activity. Similarly, compounds like 20-(S)- camptothecin and

20-(S)-9-nitrocamptothecin have antitumor potential when studied in prostate cancer, melanoma, cholangiocarcinoma, breast carcinoma, and ovarian carcinoma, while curcumin decreased lymphocytic GSTs activity (family of phase II detoxification enzymes) (Hosseini and Ghorbani 2015). Moreover, phytochemicals present in vegetables including spinach, leaf lettuce, broccoli, cabbage, cauliflower, Brussels sprouts, green beans, carrots, celery, ginger, green onions, leeks, and asparagus as well as compounds isolated from them like butylated hydroxyanisole, benzyl isothiocyanate, allyl sulfide, and dimethyl fumarate induced enzymes like GSTs, quinone reductase, and related detoxification systems followed by activating apoptosis in human colon carcinoma cells (Kirlin et al. 1999). In addition, alantolactone found in medicinal plants like *Inula racemosa*, *Inula helenium L., Inula japonica, Radix inulae,* and *Aucklandia lappa* was observed to induce detoxifying enzymes by activating PI3K and JNK signaling pathways (Millimouno et al. 2014). Similarly, phenethyl isothiocyanate, isolated from cruciferous vegetables such as watercress, modulated carcinogen-metabolizing enzyme systems. Here, phenethyl isothiocyanate induced sulfotransferase, glucuronosyl transferase, and epoxide hydrolase levels when studied in mice (Abdull Razis et al. 2014). However, UDP-glucuronosyl-transferase family of enzymes also showed the potential of detoxification of carcinogens along with clearance of anticancer drugs (Dellinger et al. 2012). Likewise, limonene and sobrerol increased phase II enzyme activities and prohibited carcinogenesis initiation in rat with breast cancer (Bruno and Njar 2007).

16.2.4.2 Enzyme Inhibition

Enzyme inhibition is a concept where the action of an enzyme is blocked. Thus, phytochemicals acting as enzyme inhibitors may block certain enzymes that are required for the cancer cell to grow. Histone deacetylases inhibitors are helpful to induce phenotypes in different transformed cells like growth arrest, mitotic cell death, apoptosis, and reactive oxygen species-assisted cell death. Here, green tea polyphenols containing a mixture of epicatechin monomers inhibited the growth of histone deacetylases activity and induced death of melanoma cells (Prasad and Katiyar 2015), while isothiocyanates, sulforaphane, and erucen phenylhexyl isothiocyanates are potent histone deacetylases inhibitors in prostate, lung, pancreatic, and bladder cancer therapy (Mitsiogianni et al. 2019). Similarly, phytochemicals like quercetin, apigenin, curcumin, indirubin, isothiocyanates, butyrate, and baicalin and baicalein (obtained from *Scutellaria baicalensis*) were reported as histone deacetylases inhibitors for anticancer therapy (Evans and Ferguson 2018). Likewise, COX-2 enzymes (convert arachidonic acid to prostaglandins) are found to be overexpressed and upregulated in many cancers. In this perspective, curcumin was found to act as COX-2 inhibitors in cell lines of colon cancer and inhibited critical stage of tumor initiation (Rahmani et al. 2014), while other compounds like tocopherols, tocotrienols, phytosterols, pterostilbene, piceatannol, and resveratrol showed their efficacy as COX-2 inhibitors (Soldati et al. 2018). Additionally, plant protease inhibitors also show their applicability in cancer therapy and have been reviewed by several authors. In this regard, protease inhibitors present in *Cicer arietinum* L as well as *Bauhinia* seeds (rich in serine and cysteine) inhibited cell viability in

prostate cancer and breast cancer, whereas *Enterolobium contortisiliquum* trypsin inhibitor blocked the activity of trypsin, plasmin, chymotrypsin, and kallikrein which leads to inhibit cell adhesion, invasion, as well as migration in gastric cancer. Moreover, seeds of *Glycine max* possess both Kunitz-type inhibitors and Bowman-Birk inhibitors which are helpful in colorectal, colon, prostate, ovarian, and breast cancers, whereas black-eyed pea trypsin/chymotrypsin inhibitor induced apoptosis in breast cancer cells (Srikanth and Chen 2016).

16.2.4.3 Enhancement of Immune Function and Surveillance

The situation when the immune system identifies and removes the cancerous cells is called immune surveillance (Cook et al. 2018). The immune system works for cancer cell removal by three steps, i.e., exclusion (elimination), equilibrium, and escape. In the exclusion type of mechanism, identification and destruction of cancer cells is taken care by the immune system. In the equilibrium type of mechanism, cancer cells are wiped out completely by the immune system. In escape type of mechanism, the immune system is helpless in controlling the proliferation of cancerous cells and hence unable to eradicate tumor (Nouroz et al. 2016). Numerous phytochemicals like isothiocyanates, genistein, lycopene, curcumin, and glucosinolates are reported to aid the regression of tumors in a few clinical trials. Furthermore, boosting the immune system was claimed by phytochemicals like flavonoids, zinc, vitamin C, and omega-3 fatty acids (Vinay et al. 2015). Some of the reviews articulate that inhibitors of the CDK-4 and CDK-6 in cancer cells were initially designed to inhibit cell cycle progression and its proliferation. But these inhibitors of CDKs proved to have an indirect effect in activation of immune surveillance. So, CDK inhibitors further studied found phytochemicals like flavopiridol, glycyrrhizin, rosamultic acid, proanthocyanidins, berberine, isoliquiritigenin, resveratrol, propolis, nar (flavanone), and indole-3-carbinol have potential role in anticancer chemotherapeutics (Bailon-Moscoso et al. 2017; Chaikovsky and Sage 2018). Enhancing natural killer cells is one of the approaches to destroy tumor by perforin and granzyme that are essential for tumor cell death as well as immune surveillance. One of the important roles of natural killer cells is to kill virally infected and cancerous cells. Phytochemicals found in *Agaricus blazei* teas, *Andrographis paniculata*, *Ganoderma lucidum*, nitrogenated soy extract, as well as natural products like ascorbic acid, immune modulator mix, transfer factor plus, selenium, and vitamin E helped to control cancer by enhancing the natural killer cells efficiency (Latorre et al. 2014; Nouroz et al. 2016). Similarly, resveratrol, polyphenols present in tea like epicatechin-3-gallate and epigallocatechin-3-gallate, improved elimination of transformed cells and enhanced innate immune surveillance (Kotecha et al. 2016). Some medicinal plant extracts or phytochemicals show promising cancer immunotherapeutic properties with different strategies and are discussed in several reviews. A recent review discusses that shikonin and hypericin helped to enhance tumor vaccine efficacy to induce immunogenic cell death in melanoma and orthotopic high-grade glioma mice models, respectively. Similarly, *Astragalus membranaceus*, *Codonopsis pilosulae*, and *Dioscorea alata var. purpurea* extract as well as polysaccharides from *Ganoderma lucidum*, *Anoectochilus formosanus*, and *Schisandra*

chinensis showed specific immune cell activation or cell-based vaccine immunity potential in the tumor microenvironment in carcinoma tumor, lung cancer patient, and melanoma mice tumor models. Moreover, artemisinin, paclitaxel, isothiocyanate, berberine, mistletoe extract, green tea extract, broccoli, resveratrol, curcumin, noscapine and its derivatives, and piperine were responsible to suppress oxidative stress in the tumor microenvironment (Yin et al. 2017).

16.2.4.4 Anti-Angiogenesis

Formation of new blood cells/vessels is termed as angiogenesis. It involves growth, differentiation, and migration of endothelial cells. Generally, tumor cells also need blood nutrients for their growth. So, chemical signals are sent by tumor cells to stimulate blood vessel growth by which it carries blood to the tumor. But, angiogenesis inhibitors (also called as anti-angiogenesis) block the nutrients and allow tumors and destroy circulating pathway for tumor cells which leads to its starvation. Hence, these angiogenesis inhibitors are important in cancer treatment (Nishida et al. 2006). Angiogenesis can be modulated by several steps such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hypoxia-inducible factor-1α (HIF-1α) signaling pathway, endothelial cell proliferation, as well as migration or MMP activity (Mirossay et al. 2018). Quercetin, cardamonin, kaempferol, luteolin, licochalcone E, rhamnazin, paxillin, epigallocatechin-3-gallate, barbigerone, galangin, myricetin, licochalcone A, chrysin, hispidulin, nobiletin, panduratin A, naringenin, xanthoangelol, xanthohumol, isoxanthohumol, eupatorin, butein, delphinidin, isoliquiritigenin, neoisoliquiritigenin, hydroxy safflower yellow A, 4′-acetoamido-4-hydroxychalcone, 1,3-diphenyl-propenone, 4-hydroxychalcone, artemisinin, taxifolin, allyl isothiocyanate, (E)-2-(4′-methoxybenzylidene)-1-benzosuberone, resveratrol, and wogonin repressed VEGF signaling pathway either because of direct effect on VEGF/VEGFR2 or by modulating VEGFR2-mediated downstream signaling. Also, chrysin, a 5,7-dihydroxyflavon, luteolin, and apigenin were found to inhibit the interleukin-6 (IL-6) pathway, while nobiletin, kaempferol, alliin, hydroxy safflower yellow A, emodin, 4-hydroxychalcone, and genistein were responsible to inhibit bFGF signaling pathway. Similarly, wogonin, astaxanthin, isoliquiritigenin, neoisoliquiritigenin, epigallocatechin-3-gallate, theaflavin-3,3′-digallate, combretastatin, oleuropein, γ-tocotrienol, nobiletin, flavopiridol, curcumin, and caffeic acid were responsible to inhibit HIF-1 signaling pathway. In addition, quercetin, xanthohumol, isoxanthohumol, curcumin, isoliquiritigenin, neoisoliquiritigenin, butein, chrysin, kaempferol, 3-hydroxyflavon, astaxanthin, nobiletin, wogonin, luteolin, thymoquinone, panduratin A, hydroxy safflower yellow A, piperine, zerumbone, myricetin, and epigallocatechin-3-gallate were found to inhibit different MMPs, specifically MMP-2 and MMP-9 (Jeong et al. 2011; Ean Jeong Seo 2013; Mirossay et al. 2018; Younas et al. 2018; Rajasekar et al. 2019).

16.3 Conclusions and Future Prospects

Unlike chemotherapeutic drugs, phytochemicals are meddling with all three stages of carcinogenesis, i.e., initiation, promotion, or progression, for protection against cancer. In addition, these phytochemicals also show a synergistic effect when used with chemotherapy medications. Additionally, they are coupled with reduced adverse effects and boosted quality and life span of the patient. Phytochemicals also help to boost the immune response of host's cells against carcinogenesis explosion. Moreover, they support to decrease, bypass, or silence molecular mechanism underlying chemoresistance including signaling cascades, cell cycle effectors, drug transporters, and nuclear transcription factors. On the other hand, plenty of outcomes regarding phytochemicals and cancer therapy from in vitro, in vivo, and preclinical trials are being carried out every day showing success to some extent. Considering this, it had been predicted that numerous cancers could be prevented just by upholding proper lifestyle alterations, while interfaces of the phytochemicals as cancer medication may uncover some economic, safe, and nontoxic anticancer therapeutic in the future. Besides, further efforts are justifiable to appreciate potency, metabolism, stabilities, polymorphism, toxicities, drug–drug interactions, pharmacokinetics, and dynamics, along with the formulations, degradation, and dosages regimens of phytochemicals.

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Anticancer and Chemopreventive Phytochemicals from Cruciferous Plants

17

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Contents

Abstract

Cruciferous veggies are a varied group of vegetables of the family, brassicaceae that includes cauliflower, broccoli, cabbage, Brussels sprouts, bok choy, kale, arugula, etc., and play a vital part in the human diet. Apart from being a good

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source of nutrients, they contain various natural compounds that are valuable for human health. Consuming cruciferous veggies can, astonishingly, be helpful in the chemoprevention of cancers. Cruciferous plants contain many bioactive natural products like polyphenols, flavanoids, isothiocyanates, lignans, phytosterols, carotenoids, and indole-3-carbinol. The most studied bioactive phytocompounds found in cruciferous veggies include glucosinolates and indole-3-carbinol. Brassica vegetables with glucosinolates and their hydrolysis products exhibit several biological properties like antioxidant, chemopreventive, and anticarcinogenic properties. In addition, they are found to be nontoxic with negligible adverse effects. Isothiocyanates (ITCs) and indoles inhibit carcinogenesis in various organs of mice and rats, including the breast, urinary bladder, liver, lung, colon, and stomach. Likewise, sulforaphane of cruciferous plants is found effective as antioxidant, anticancer, and chemopreventive agent. These compounds safeguard cells from DNA damage, induce apoptosis, nullify carcinogens, and inhibit angiogenesis and migration of tumor cells. In vitro and in vivo experiments have disclosed various potential pathways through which these compounds prevent cancer. This chapter aims to highlight the anticancer and chemopreventive effects of various phytochemicals isolated from cruciferous plants.

Keywords
Chemoprevention · Cancer · Disease · Sulforaphane · Brassinosteroids · Bioactive compounds

17.1 Introduction

Chemoprevention is defined as "the use of non-cytotoxic nutrients or pharmacological agents to enhance physiological mechanisms that protect the organism against mutant clones of malignant cells" (Morse and Stoner 1993). Cancer chemoprevention is achieved by using naturally or synthetically derived molecules to inhibit, interrupt, or reverse carcinogenesis before it leads to malignancy. Various preclinical and epidemiological studies have led to the identification of a varied range of naturally occurring compounds and dietary substances with significant chemopreventive effects. In vitro and in vivo experiments endorse that phytochemicals may modulate various signaling pathways associated with cell multiplication and apoptosis in cancerous cells, boosting cellular immunity, and sensitizing cancer cells to facilitate the action of antiproliferative agents (Kotecha et al. 2016). A relationship between health and diet has attracted attention for centuries, but a link between diet and cancer has attracted attention only in recent years. Consumption of cruciferous vegetables has been linked with prevention of risk from various cancers, especially cancers of the lung, gastrointestinal tract, and prostate (Razis and Noor 2013). These vegetables contain diverse health promoting natural compounds including ascorbic acids, folic acids, phenolics, carotenoids, glucosinolates (GSs),

and brassinosteroids (BRs) which offer effective broad-spectrum protection against the cancer-stimulating agents encountered in our everyday life. Unlike vegetables from other plants, cruciferous veggies have considerable quantities of sulfur-comprising GSs, which, on hydrolysis, by the enzymatic action of myrosinase or enzymes of few intestinal microbes, are transformed into bioactive molecules, such as isothiocyanates (ITCs) and indoles (Shapiro et al. 2006; Becker and Juvik 2016). These phytochemicals are thought to be responsible for the chemopreventive effects offered by higher consumption of vegetables and fruits from the cruciferous plants. Plant secondary metabolites are one of the most promising bioactive molecules for cancer prevention. This chapter aims to highlight the anti-carcinogenic and chemopreventive effects of various phytochemicals isolated from cruciferous plants.

17.2 Important Cruciferous Vegetables

Vegetables obtained from the plants, belonging to the family of *Brassicaceae* are commonly recognized as cruciferous vegetables. These vegetables include arugula or rocket (*Eruca sativa*), bok choy (*Brassica rapa* subsp. chinensis), broccoli (*Brassica oleracea* var. *gemmifera* and *B. oleracea* var. Italic), cabbage (*B. oleracea* var. capitata), cauliflower (*B. oleracea* var. botrytis), collard greens, horseradish (*Armoracia rusticana*), radishes (*Raphanus raphanistrum* subsp. sativus), kale (*B. oleracea* var. sabellica), Rutabaga (*B. napobrassica*), turnips (*B. rapa* subsp. Rapa), watercress (*Nasturtium officinale*), and wasabi (*Eutrema japonicum*) (Fig. 17.1).

17.3 Glucosinolates (GSs or GLs)

Glucosinolates (GSs or GLSs) are a group of sulfur-rich, amino acid derived phytometabolites occurring in the plants of *Brassicaceae* family. Various GSs like sinigrin, gluconapin, glucobrassicanapin, progoitrin, epiprogoitrin, napoleiferin, glucoiberin, glucoraphanin, glucoalysin, gluconasturtiin, glucobrassicin, 4-OH glucobrassicin, 4-OMe glucobrassicin, and neoglucobrassicin have been evaluated in edible parts of *B. oleracea* (Brussels sprouts, broccoli, kale, cabbage, and cauliflower) (Fig. 17.2). Broccoli is an important source of glucoraphanin, while cabbage possesses higher levels of sinigrin. Likewise, watercress (*Nasturtium officinale*) is rich in gluconasturtiin content, which is a type of Gas, and most likely imparts pest-inhibiting property to growing crucifers. There are more than one hundred fifty known GLs, and all of them share a general sulfur-allied β-D-glucopyranose skeleton; however they vary in the nature of the substituent or side chain R. Side chains of these GLs are from various amino acids during their biosynthesis in cruciferous plants. These GLs are classified into various subgroups on the basis of the molecular arrangement of the side chains (R). For example, the alkylthioalkyl chain of glucoraphanin has a sulfur containing functionality (sulfinyl group), while the aryl or aromatic side chain of gluconasturtiin is a phenethyl substituent (Fig. 17.2) (Navarro et al. 2011).

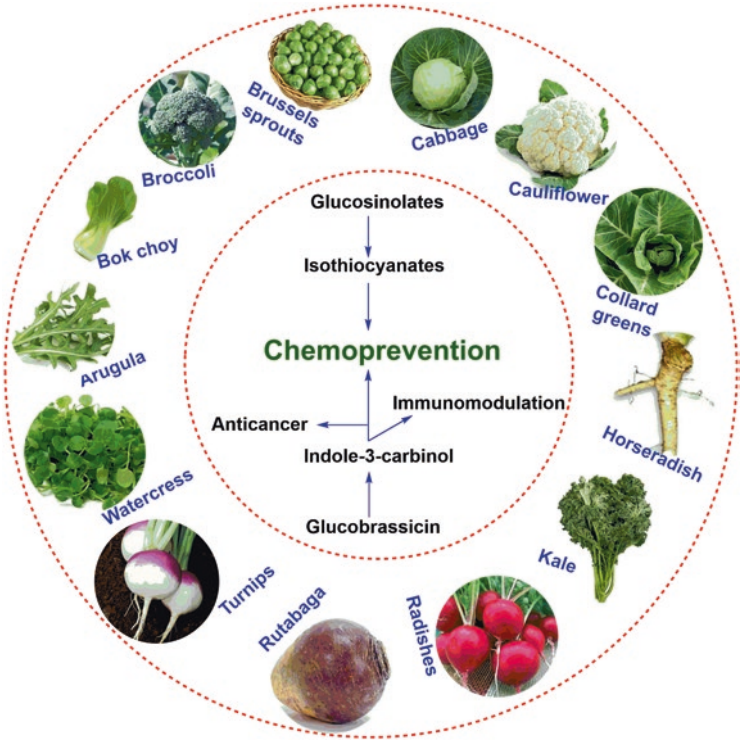

Fig. 17.1 Important cruciferous vegetables and their chemopreventive effects

17.4 Hydrolysis Products of Glucosinolate and Their Chemopreventive Effect

Crucifers contain many biologically active components; glucosinolates (GLSs) are one such important components which have been extensively investigated. These GLSs undergo enzyme-catalyzed hydrolysis and yield different hydrolytic products like ITCs and various others products like epithionitriles, indoles, thiocyanates, nitriles, and oxazolidine-2-thione. Out of various hydrolytic products of GLSs, ITCs are strongly linked with cancer chemoprevention of various tissues or organs in human beings (Singh and Singh 2012). Many numbers of in vitro and in vivo experiments propose that ITCs exert their biological effects through diverse interconnected signaling pathways, including detoxification, apoptosis, and cell cycle regulation, which are essential for inhibition of carcinogenesis. The effects of ITCs on human health have been broadly evaluated, and these hydrolytic products are involved in the anti-carcinogenic effects of these cruciferous vegetables (Verhoeven et al. 1997). Glucoraphanin of broccoli is hydrolyzed to sulforaphane (Kushad et al. 1999) (Fig. 17.3). Sulforaphane has been recognized as an important anticancer ITC in humans, which induced apoptosis in tumor cells

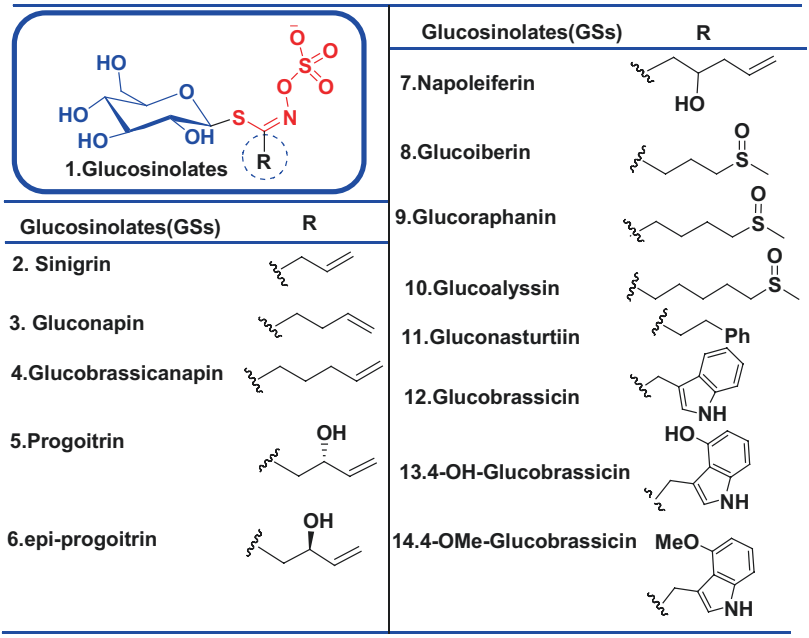

Fig. 17.2 Various glucosinolates in cruciferous vegetables share a common sulfur-linked β-D-glucopyranose structure, but varies in side chain R

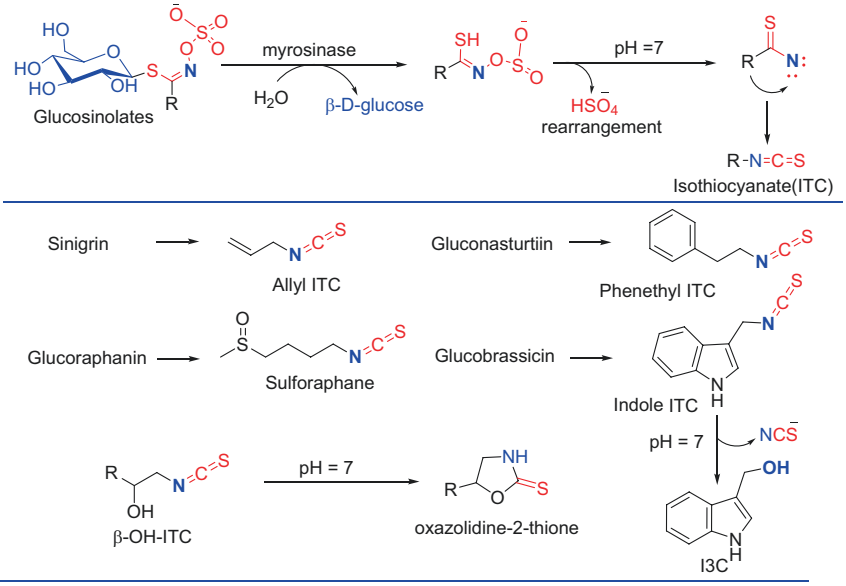

Fig. 17.3 Enzymatic conversion of glucosinolates to their corresponding isothiocyanates (ITCs)

(Gamet-Payrastre et al. 2000) and inhibited the rate of tumor growth in various model organisms (Zhang et al. 1994; Chung et al. 2000).

The biological activities of hydrolysis products of glucosinolates (GSHPs) linked to cancer chemoprevention in human beings have been supported by various researches. The crucial mechanism of chemoprevention provided by GSHPs is by modulating phase I/phase II enzymes and inducing various antioxidant enzymes, such as heme oxygenase 1, NAD(P)H quinone reductase, and glutathione S transferases via the Keap1-Nrf2-ARE signaling. The stimulation of this molecular pathway is commonly linked with aliphatic ITCs, while few indole-based GSHPs have also been linked with the stimulation of antioxidant enzymes (Becker and Juvik 2016) (Fig. 17.4).

17.5 Sulforaphane (SFN) and Its Chemopreventive Effect

Common cruciferous ITCs, such as sulforaphane (SFN) and phenethyl isothiocyanate (PEITC), have been confirmed to inhibit carcinogenesis via inducing cancerous cell growth apprehension and modulating apoptosis in various cancers, including skin (Xu et al. 2006), bladder (Munday et al. 2008), colon (Gamet-Payrastre et al. 2000), breast (Li et al. 2010), ovary (Chuang et al. 2007), blood (Suppipat et al. 2012), and prostate cells (Singh et al. 2004). The mechanism by which ITCs achieve this assignment is not well explained and perhaps not widely accepted, but a few of the well-known effects of ITC-treated cells include alternative gene splicing and modulation of gene expression (Traka et al. 2010). Perhaps more significantly, many ITCs have revealed to improve the functioning of Nrf2 (the nuclear factor erythroid 2-related factor 2) (Saw et al. 2011). After activation, Nrf2 encourages the rate of transcription antioxidant enzymes and phase II genes that eventually help

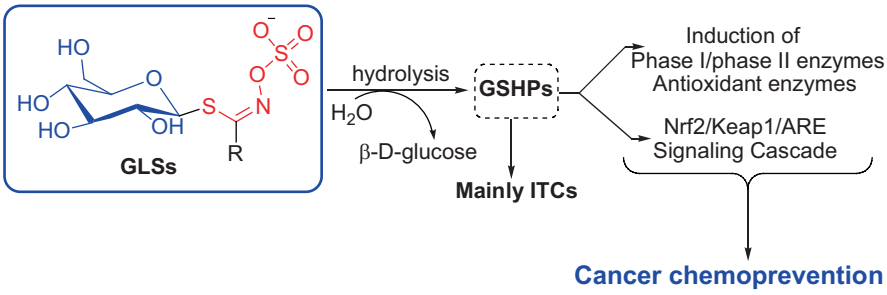

Fig. 17.4 Mechanism of chemoprevention by glucosinolate hydrolysis products, mainly ITCs. PII enzymes: UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), glutathione S-transferases (GSTs), N-acetyltransferases (NATs), and S-and O-methyltransferases (MTs). **PI enzymes**: many cytochrome P450s (CYPs). **Antioxidant (AO) enzymes:** catalases (CAT), superoxide dismutases (SOD), glutathione reductases (GSR), glutathione peroxidases (GPX), glutaredoxins (GLRX), thioredoxins (TXN), thioredoxinreductases (TXNRD), heme oxygenase 1 (HO-1), and NAD(P)H:quinoneoxidoreductase 1 (NQO1)

cells to overcome cancer development (Surh 2003; Zhang and Gordon 2004). It appears that most ITCs from crucifer vegetables induce phase II enzymes; however there might be disparity amongst aromatic ITCs and aliphatic ITCs in their modulatory activities of phase I enzymes (Leibelt et al. 2003; Jeffery and Araya 2009; La Marca et al. 2012).

Sulforaphane (SFN) exhibit its anti-carcinogenic effects via modulation of key genes and signaling pathways involved in cell cycle blocking and induction of apoptosis in different cancer cells. Various researches on the molecular mechanisms of the antitumor effects of sulforaphane have revealed that SFN might reverse epigenetic changes in different cancer cells by affecting various enzymes like histone deacetyltransferases, DNA methyltransferases, and noncoding RNAs (Su et al. 2018). In basal environment, Keap1 gene binds to Nrf2 gene, which promotes degradation of proteasomes by ubiquitination. Due to oxidative stresses, Nrf2 gene detaches from Keap1 gene, and later it gets translocated into the nucleus of the cells, where it interacts with the promoter sites of the target genes. This signaling carries out the expression of various cytoprotective genes, such as heme oxygenase and superoxide dismutase. In TRAMP C1 (prostate cancer) cells, sulforaphane can check the expression and action of various enzymes, such as DNA methyltransferases and histone deacetylases. A significant inhibition of these enzymes is also being identified in tetradecanoylphorbol acetate-stimulated mouse epidermal skin (JB6 P+) cells treated by sulforaphane. The compound alleviated the CpG methylation and increased histone acetylation of the Nrf2 gene. Eventually, this epigenetic modulation by sulforaphane promoted the transcription, nuclear translocation, and activation of Nrf2 gene (Su et al. 2018) (Fig. 17.5).

17.6 Indole-3-Carbinol (I3C) and Its Chemopreventive Effect

Indole-3-carbinol (I3C) is a derivative of glucobrassicin hydrolysis by the enzymes, which is found exclusively in various cruciferous veggies, including radish, cabbage, broccoli, daikon, Brussels sprouts, and cauliflower (Broadbent and Broadbent 1998). The hydrolysis of glucobrassicin by the enzyme (myrosinase) at pH 7 produce 3-indomethyl ITC, which further converts to thiocyanate ion and indole-3-carbinol. During cooking of cruciferous vegetables, myrosinase is denatured and thus the hydrolysis of glucosinolates is checked. Non-hydrolyzed glucosinolates then transport to the colon and are metabolized by human intestinal bacteria. The formation of I3C from glucobrassicin may still occur to a smaller degree in the large intestine due to the enzymatic action of myrosinase of colonic bacteria (Barba et al. 2016).

I3C has been revealed to suppress the multiplication of several types of human cancer cells, including breast, colon (Howells et al. 2002; Frydoonfar et al. 2002; Rahman et al. 2003; Hudson et al. 2003), and prostate cancer cells (Frydoonfar et al. 2003; Nachshon-Kedmi et al. 2003). I3C has a remarkable potential of preventing cancers, and it exerts its effects via various modes of actions. Interestingly, it offers

cytoprotective activity for the normal cells; however it functions as a unique apoptotic agent for cancerous cells. This valuable potential of indole-3-carbinol should be further investigated in combination cancer therapy with various chemotherapeutic agents (Fig. 17.6).

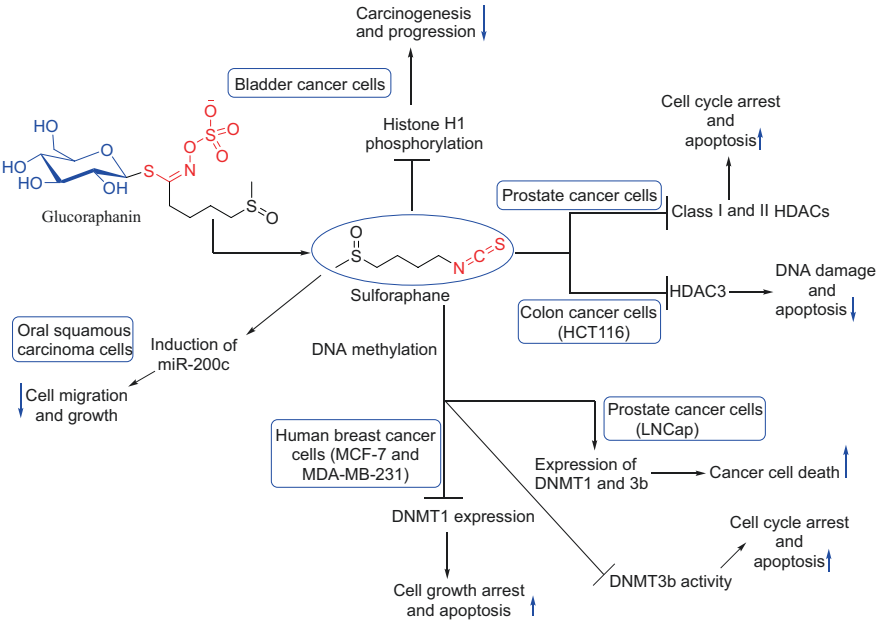

Fig. 17.5 Anticancer effects of sulforaphane (SFN) against various cancers (based on Su et al. 2018)

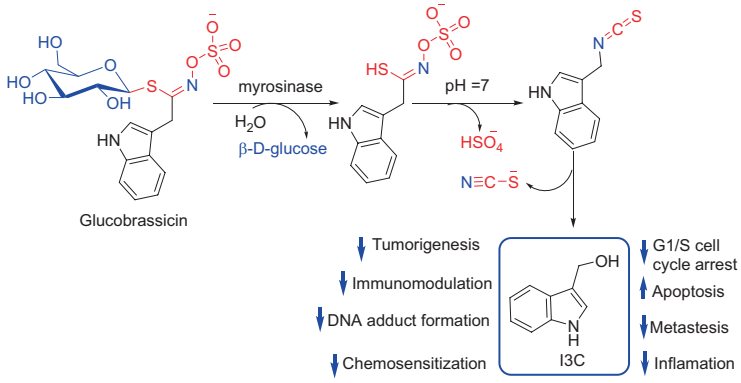

Fig. 17.6 Biological actions of indole-3-carbinol

17.7 Brassinosteroids (BRs) and Its Chemopreventive Effect

Brassinosteroids (BRs), a unique group of plant-based steroidal hormones that are necessary for plants growth and development, provide resistance and tolerance against disease stress and regulation of senescence (Bishop and Koncz 2002). Brassinosteroids affect vegetal development via various physiological responses. Studies have revealed their activities against stress, viruses, human cancers, and genotoxic effects. The most common naturally occurring brassinosteroids, 24-epibrassinolide and 28-homocastasterone (Fig. 17.7), were investigated by different research groups to evaluate their anticancerous activity. BRs were investigated against various cancer cells including CEM, multiple myeloma RPMI 8226, T-lymphoblastic leukemia, cervical carcinoma, A-549, lung carcinoma, HeLa, osteosarcoma HOS cell lines (Malikova et al. 2008), breast cancer, and prostate cancer cells. The outcome of their studies has revealed that BRs can induce apoptosis by interacting with the cell cycle (Malikova et al. 2008; Steigerova et al. 2010, 2012). BRs may targets ER (estrogen receptor), EGFR (epidermal growth factor receptor), and HER-2 (human EGFR-2) proteins, which are essential for the treatment of breast cancer as they are abundant in breast cancer cells, such as MCF-7, T47D, MDA-MB-468, and MDA-MB-231 (Pledgie-Tracy et al. 2007; Steigerova et al. 2010, 2012). Treatment of 28-homocastasterone and 24-epibrassinolide with breast cancer cells showed reduction in cyclin proteins which are involved in G_1 phase of the cell cycle. The treatment of prostate cancer cells with these BRs induces programmed cell death by increasing levels of the pro-apoptotic protein (Bax) and reduction of anti-apoptotic protein (Bcl-2) (Steigerova et al. 2012).

17.8 Conclusions

Cruciferous vegetables are important sources of various phytochemicals that have remarkable inhibitory effects on pathways of carcinogenesis for different cancers, due to their antiproliferative and chemopreventive properties. In vitro and in vivo experiments have disclosed various potential pathways through which these phyto compounds prevent cancer. The consumption of these vegetables is advantageous in the sense that they are sources of glucosinolates which on enzymatic action give rise to isothiocyanates like sulforaphane and indole-3-carbinol. In addition, significant inhibition of uncontrolled cell growth as well as induction of apoptosis has been reported with the integration of indole-3-carbinol and indole-3-carbinols. However,

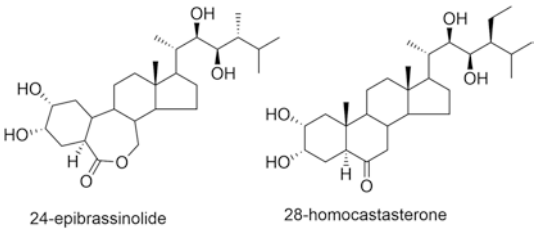

Fig. 17.7 Structures of some anticancer brassinosteroids (BRs)

clinical studies, carried out till now on the role of cruciferous vegetables in preventing cancers in humans have shown mixed results, and further research is needed to conclusively establish the use of cruciferous veggies for cancer prevention in humans.

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Pharmacological Properties of Essential Oil Constituents and their Mechanisms of Action

18

Bibi Sharmeen Jugreet
and Mohamad Fawzi Mahomoodally

Contents

Abstract

Since time immemorial, essential oils (EOs) have been utilized for their therapeutic properties. However, during recent decades, a renewed interest has been experienced in EOs and their individual constituents due to their remarkable pharmacological potentials evidenced by various experimental studies. For instance, they are broadly acknowledged for their antimicrobial, antidiabetic, anti-inflammatory, antispasmodic and anticancer properties. Also, EOs have demonstrated to improve conditions associated with brain and cardiovascular diseases amongst others. Multiple studies have identified and isolated EOs phytocomponents possessing therapeutic activities. In addition, studies have elucidated their underlying mechanisms of actions involved in the treatment and management of several ailments.

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Thus, given the wide spectrum of biological activities exhibited by these active compounds, this chapter endeavours to provide a mechanistic overview of some common EOs constituents with regard to their pharmacological properties.

Keywords
Essential oils · Major · Active · Components · Biological activities · Mechanism of actions

18.1 Introduction

Essential oils (EOs) are volatile aromatic, hydrophobic and oily liquids obtained from plants. They may be produced from cells or groups of cells specialized within the specific parts of plants, such as leaves, bark or wood, foliage, fruits, seeds, stems and rhizomes (Miguel 2010). EOs play a significant role in protecting the plants against bacterial, viral, fungal and insect attacks, and also herbivores by decreasing their appetite for the plants. Besides, they serve in attracting some insects for seeds and pollen dispersal or help to keep away unwanted insects (Bakkali et al. 2008).

EOs can be obtained by a number of different techniques, including fermentation, expression or enfleurage, although hydrodistillation is the most commonly used one (Speranza and Corbo 2010). A large number of herbal plants have been studied for their EOs and exploited for commercial applications (Mohammadreza 2008), particularly in cosmetics, perfumery, agriculture and food industries (Burt 2004).

EO constituents can be categorized into two distinct classes of chemicals, namely terpenes and phenylpropanoids. Even though terpenes and their derived oxygenated compounds (terpenoids) are more common and rich in EOs, certain species are composed of high amounts of shikimates, notably phenylpropanoids, which confer a specific flavour and odour to the plant (Tisserand and Young 2013; Baser and Buchbauer 2015; Zuzarte and Salgueiro 2015). However, it is important to note that EOs composition can vary depending on factors, such as variety of plants, parts of the plant, growth area, time of harvest, climatic changes, conditions of storage as well as the chemotype of each constituent (Pauli and Schilche 2009).

EOs and their active components have been extensively studied for their vast range of biological activities along with their underlying mechanisms of actions. For instance, they have been reported to act as natural antimicrobials (Edris and Farrag 2003; Burt 2004; Swamy et al. 2016) and have even been evaluated for their potential uses as substitute antidotes for the treatment of several infectious diseases (Freires et al. 2015). Additionally, they are regarded as potent antioxidants, anti-inflammatory (Miguel 2010; Pérez et al. 2011) and antidiabetic agents (Tahir et al. 2016; Ya'ni et al. 2018), amongst others. EOs have also demonstrated anticarcinogenic effects through a range of mechanisms, including acting directly on the tumour cells or by interaction with the microenvironment (Edris 2007;

Sitarek et al. 2017). Moreover, their importance in aromatherapy has been widely documented. For instance, in medical aromatherapy, EOs have been reported to help in health promotion and treatment of clinically diagnosed medical diseases (Maeda et al. 2012).

Even though EOs major constituents are crucial for their bioactivities, minor components can play a significant role as well, since they can increase the effects of the major constituents. However, some of these EOs compounds have been shown to exhibit both additive and antagonistic effects (Bassolé and Juliani 2012). Therefore, EOs bioactivity can be regarded as the sum of its components acting either synergistically or antagonistically (Baser and Buchbauer 2010; Elshafie et al. 2015). This chapter aims to discuss different mechanisms of actions demonstrated by EOs constituents (in synergism, as single agents or as potentiating agents in tandem with conventional drugs) in relation to their pharmacological properties.

18.2 Essential Oils (EOs) Major Compounds and Their Mechanisms of Actions

18.2.1 Antimicrobial Property

The antimicrobial potentials of EOs and their components have been extensively studied in the search for more effective antimicrobials to combat antibiotic-resistant pathogenic microorganisms (Swamy et al. 2016). In that aspect, EOs that are abundant with phenols or aldehydes, such as cinnamaldehyde, citral, thymol, eugenol or carvacrol (Fig. 18.1) were found to exhibit the highest antibacterial potential, followed by those composed of terpene alcohols like terpineol, fenchyl alcohol, and borneol. On the other hand, volatile oils consisting of esters or ketones like geranyl acetate, α-thujone or β-myrcene demonstrated much weaker activity, while EOs containing terpene hydrocarbons were generally not active (Davidson 1997; Dorman and Deans 2000). Besides, EOs rich in phenolic compounds like eugenol, carvacrol and thymol (Fig. 18.1) are usually characterized by significant antibacterial activities (Knobloch et al. 1986; Dorman and Deans 2000; Lambert et al. 2001; Swamy et al. 2016). In addition, these compounds have been held accountable for the disruption of plasma membrane, protons driving force, active transport, electron flow as well as the coagulation of cellular contents (Sikkema et al. 1994; Denyer and Hugo 1991; Pauli 2001; Swamy et al. 2016). Thus, the antimicrobial activity of EO constituents is determined by the lipophilicity of their hydrocarbon skeleton as well as the hydrophilicity of their major functional groups, and accordingly it has been classified in the following order: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons (Kalemba and Kunicka 2003).

A major compound of EOs, eugenol (Fig. 18.1) has been evaluated for its antibacterial effect and its mechanism of action against *Salmonella typhi* has been elucidated (Devi et al. 2010). According to them, eugenol was observed to decrease the viability of cells and cause absolute inhibition of the bacterium with the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of

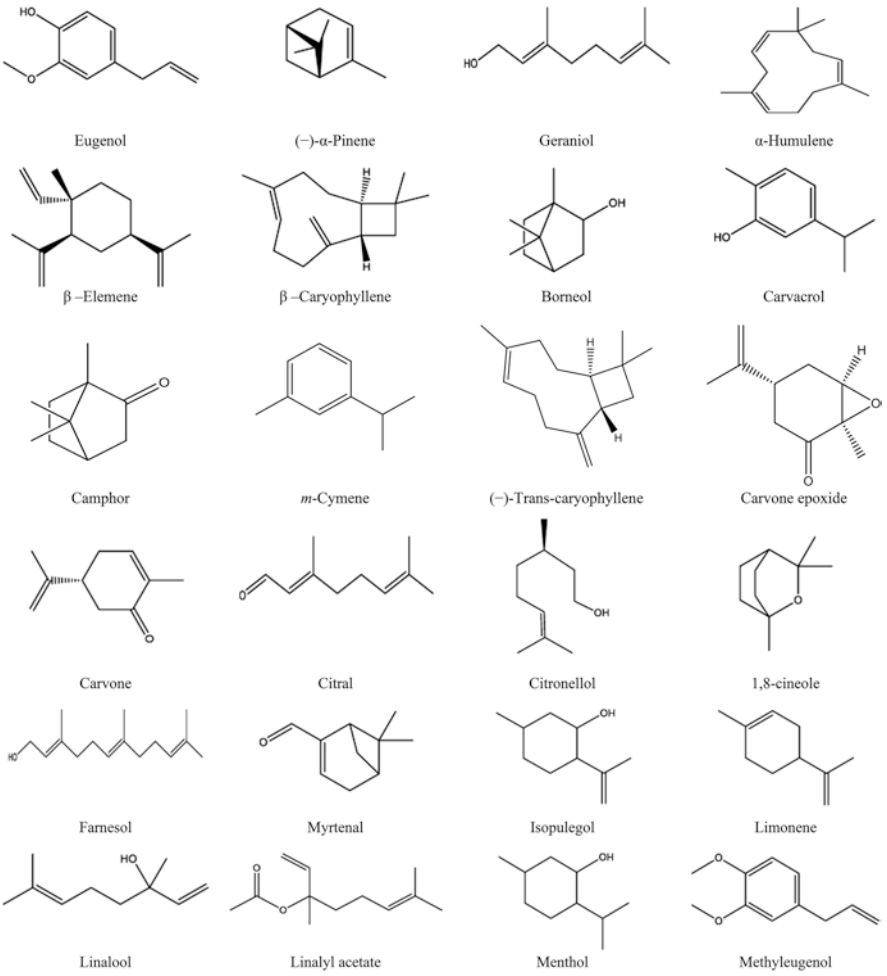

Fig. 18.1 Chemical structures of EO constituents discussed in book chapter

0.0125% and 0.025%, respectively. Additionally, inactivation of *S. typhi* was induced within 60 min of exposure to eugenol. Eugenol's chemoattractant property along with the observed strong antibacterial activity at alkaline pH suggests that this compound can act more effectively when given in vivo. Furthermore, the interaction of eugenol on *S. typhi* cell membrane was found to be responsible for its antibacterial action. In this regard, eugenol was noted to cause increased membrane permeability, disruption of bacterial cell membrane and distortion of membrane macromolecules. Likewise, other studies have confirmed the antibacterial activity of eugenol to be indicative of its disruptive action on cytoplasmic membrane, thus enhancing its non-specific permeability (Gill and Holley 2006; Swamy et al. 2016). It has also been

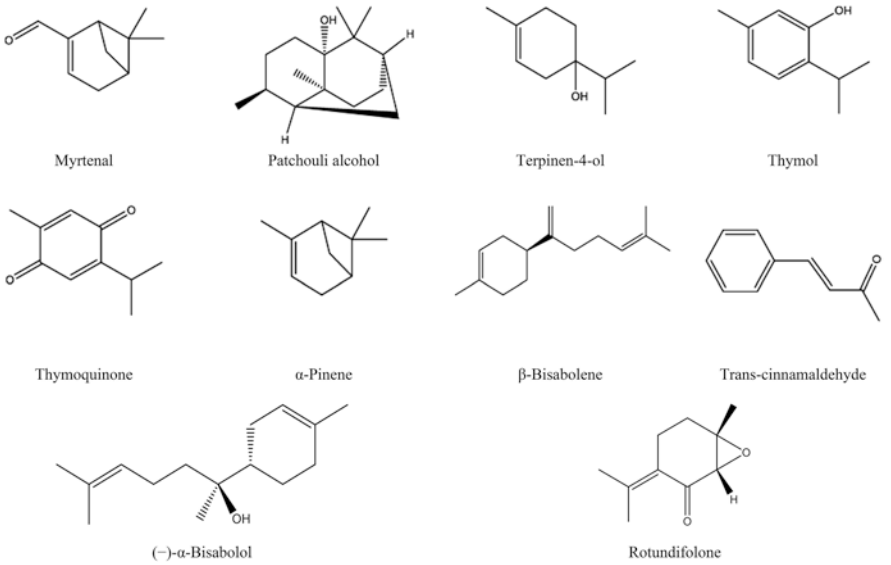

Fig. 18.1 (continued)

highlighted that the hydrophobic characteristic of eugenol permits its penetration in the lipopolysaccharide of the gram-negative bacterial cell membrane, hence modifying the structure of the cell and resulting into the escape of intracellular components (Burt 2004). Also, the hydroxyl group of eugenol is believed to attach to proteins, which then prevents enzyme action in *Enterobacter aerogenes* (Burt 2004).

The mechanism of action of (−)-α-pinene (Fig. 18.1) in relation to its modulation of antibiotic resistance in *Campylobacter jejuni* has also been reported by Kovač et al. (2015). Remarkably, (−)-α-pinene was found to effectively modulate antibiotic resistance in *C. jejuni* by reducing the MIC of erythromycin, triclosan and ciprofloxacin by up to 512-fold. Further, they used insertion mutagenesis method using ethidium bromide to identify the target antimicrobial efflux systems, and DNA microarrays were used to evaluate *C. jejuni* adaptability to (−)-α-pinene. The results showed that (−)-α-pinene promoted the expression of cmeABC as well as Cj1687, a putative antimicrobial efflux gene. The accumulation of ethidium bromide was found higher in the wild-type strain as compared to the antimicrobial efflux mutant strains. Thus, it confirmed that these antimicrobial efflux systems were the target of action of (−)-α-pinene. Furthermore, a decrease in membrane integrity induced by (−)-α-pinene implied that the enhanced microbial influx was the secondary mode of action of (−)-α-pinene. It was also revealed from the findings that (−)-α-pinene caused the disruption of several metabolic pathways, in particular those implicated in heat-shock retorts. Therefore, the inhibition of microbial efflux and reduced membrane integrity including metabolic disruption were amongst the mechanisms of (−)-α-pinene involved in the modulation of antibiotic resistance in *C. jejuni*.

Kannappan and co-authors (2019) demonstrated the combinatorial antibiofilm efficacy of geraniol (Fig. 18.1) and cefotaxime against *S. epidermidis* (ATCC 35984) and MRSA (ATCC 33591). Cefotaxime, a broad-spectrum third-generation cephalosporin antibiotic, acts by binding to one or more of the penicillin-binding proteins and hence causes inhibition of bacterial cell wall formation (Luthy et al. 1979). Significant diminution in biofilm biomass and slime formation was observed following treatment with geraniol and cefotaxime combination (GCC), yielding a minimal biofilm inhibitory concentration of 100 μg/mL and 2 μg/mL of geraniol and cefotaxime respectively. The results also revealed that GCC targeted the initial attachment of the cells associated with biofilm formation. Moreover, treatment of the test pathogens to GCC reduced the production of staphyloxanthin pigment in MRSA and consequently rendered the pathogenic cells vulnerable to the host immune responses. In addition to inhibiting biofilm formation, GCC was found to suppress the production of extracellular polymeric substance (EPS) and slime. GCC treatment was also found to induce reduced expression of surface adhesin genes, and in MRSA, the gene responsible for the production of virulence factor such as staphylococcal enterotoxin A was downregulated. Microscopic analysis made on GCC-treated EPS corroborated with the findings of the in vitro biofilm inhibition assay, thus establishing the destructive effect of GCC on the evaluated pathogens' biofilm formation. Besides, GCC considerably increased the vulnerability of the test pathogens towards human blood. In vivo assay conducted on *Caenorhabditis elegans* also revealed the antibiofilm potential of GCC in the control of biofilm-associated infections caused by *Staphylococcus* species. For instance, while the nematodes exposed to the test pathogens demonstrated an enhanced colonization together with deformed pharynx and internal hatching of eggs, those treated with GCC and test pathogens cells displayed reduced internal colonization as well as healthy pharynx (Kannappan et al. 2019).

Moreover, Yuan and Yuk (2019) investigated the adaptive responses of *E. coli* O157:H7 with regard to its virulence gene expression and virulence properties at the sublethal concentrations of trans-cinnamaldehyde (TC) (Fig. 18.1), carvacrol (Car) (Fig. 18.1) and thymol (Thy) (Fig. 18.1). *E. coli* O157:H7 grown to the early stationary phase in the presence of sublethal EOs demonstrated notable reduction in motility (reversible subsequent to stress elimination), biofilm formation capacity and efflux pump activities, without inducing antibiotic resistance or infection, since no marked changes were noted with regard to the invasive and adhesive capacity of the test pathogens on human colon adenocarcinoma (Caco-2) cells. Reduced expression of related virulence genes, together with those encoding biosynthesis and functioning of flagella, biofilm formation regulators and multidrug efflux pumps, in addition to type III secretion system components, was reported. Hence, TC, Thy and Car at sublethal doses did not seem to cause potentiation of virulence in adapted *E. coli* O157:H7 (Yuan and Yuk 2019).

Further, Guimarães et al. (2019) investigated the antibacterial activity of several terpenoids and terpenes found in EOs. In general, the results showed that oxygenated functional groups in terpenes showed better antibacterial action compared to hydrocarbons. Out of 16 compounds identified to possess antibacterial activity,

carvacrol, eugenol and thymol were found to be the most potent; even greater than sulfanilamide against the four strains tested (*B. cereus, E. coli, S. aureus* and *S. typhimurium*). Contrastingly, compounds such as borneol, camphor, *m*-cymene, (±)-linalool and (+)- and *R*-(+)-limonene (Fig. 18.1) displayed the least antibacterial activity. Nonetheless, only six of the 16 compounds that showed antimicrobial action were seen to be bactericidal (absence of growth) in the study. For instance, none of compounds tested were found to be bactericidal against *B. cereus*, while only terpineol and thymol demonstrated bactericidal activity against *S. aureus*. However, the lowest MBC value against *S. typhimurium* (0.06 mg/mL) was presented by eugenol. Moreover, although thymol exhibited the lowest MIC values against the tested strains, it did not show any bactericidal action at concentrations of MIC, 2× MIC as well as 4× MIC, but rather at concentration 0.12 mg/mL. Eugenol and thymol were observed to cause inhibition (IC_{100}) of *S. aureus* and *S. typhimurium* growth. The morphological changes detected in *E. coli, S. aureus* and *S. typhimurium* that were treated with β-citronellol, L-carveol and trans-geraniol were also analysed. The treated *E. coli* cells were of irregular sizes with the presence of debris, probably due to interrupted cell division or cell membrane dysfunction compared to the control cells which had smooth surfaces and bacillary shapes. Besides, cells treated with geraniol and citronellol were smaller, had considerably rough surfaces and the cells were adhered to one another. On the other hand, *S. aureus* treatment with terpineol disrupted cell division and were found to have a distorted "grape bunch" shape, a typical morphology of the colonies. As for *S. typhimurium*, terpineol and eugenol treatment revealed the loss of cell membrane integrity or function as the death mechanisms involved, whereby the cell membrane was completely destroyed along with the presence cell debris. Further, carveol, citronellol, eugenol, geraniol and terpineol were found to be fast-acting compounds, given that they caused *E. coli* and *S. typhimurium* inactivation within 2 h. The authors also pointed out that the compounds that exhibited the best activity in relation to their MIC and time-kill kinetics had low molecular weights as well as polar functional groups. Such features could enhance antimicrobial potential by facilitating permeation via the outer cell membrane as confirmed by eugenol action, a low-molecular-weight phenolic compound that displayed fast time-kill kinetics leading to the death of *S. typhimurium* at all concentrations in only 2 h (Guimarães et al. 2019).

Carvacrol (Fig. 18.1), a major monoterpene of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) volatile oils, has been reported to possess a strong antifungal activity against *Candida albicans* (Chaillot et al. 2015). In the attempt of understanding the underlying mechanism of action, they demonstrated that fungal cells need the UPR (unfolded protein response) signalling path to be able to counterattack carvacrol. Moreover, carvacrol was found to act as an ER (endoplasmic reticulum) stress inducer, disturbing the morphology as well as the integrity and protein-folding capacity of the ER leading UPR activation. Carvacrol also enhanced the antifungal action of fluconazole, echinocandin and caspofungin (antifungal drugs) as well as UPR inducers, such as tunicamycin and dithiothreitol against *C. albicans*.

Furthermore, Xia et al. (1999) demonstrated in their study that α-pinene (Fig. 18.1) exerts a significant antifungal activity against *C. albicans*. The

antifungal mechanisms that were put forward included the rupture of *C. albicans* cell wall and cytoplasmic membrane, the release of intracellular components and fusion of cell residues into irregular masses. Besides, there was the inhibition of RNA, DNA, cell wall polysaccharide and cell membrane ergosterol synthesis.

Linalool and geraniol (Fig. 18.1), two major oxygenated monoterpenes present in EOs of various medicinal plants such as *Pelargonium graveolens*, *Peperomia pellucida* and *Acorus calamus* (Souza et al. 2016; Okoh et al. 2017; Parki et al. 2017), have also been tested for their anticandidal potential against five *Candida* species of ATCC strains (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. tropicalis*) (Singulani et al. 2018). Although both linalool and geraniol were found to inhibit candidal growth in vitro, geraniol was observed to be more effective. Furthermore, while *C. albicans* was mostly resistant to the compounds (MIC $\geq$ 1000 μg/mL), *C. parapsilosis* was susceptible (MIC 37.5 and 125 μg/mL for geraniol and linalool, respectively) (Singulani et al. 2018).

18.2.2 Antidiabetic Property

Citronellol (Fig. 18.1), which is a monoterpene alcohol commonly found in citrus species, such as oranges, lemons, and pomelos, has been evaluated for its antidiabetic effect in streptozotocin (STZ)-induced diabetic rats (40 mg/kg body weight (b.w.)) (Srinivasan and Muruganathan 2016). According to them, citronellol administered orally for 30 days (50 mg/kg) was observed to improve the insulin, hepatic glycogen and haemoglobin levels along with a substantial decline in the levels of glucose and glycated haemoglobin (HbA1c). Additionally, changes in enzyme activities involved in carbohydrate metabolism, kidney and hepatic biomarkers were brought to near-normal levels in citronellol-treated rats. Hypoplasia and extensive damages of the islets of langerhans in STZ-induced diabetic rats were decreased in groups treated with citronellol. Moreover, citronellol supplement enhanced insulin immunoreactivity and augmented the number of immunoreactive β-cells in the treated group. Thus, citronellol demonstrated the capacity to improve the secretion of insulin or regeneration of the β-cells in the diabetic animals (Srinivasan and Muruganathan 2016).

The effect of carvone (Fig. 18.1), a monoterpene ketone, on carbohydrate metabolic enzymes in the liver of STZ-induced diabetic rats (40 mg/kg b.w.) was also studied in a similar way by Muruganathan and Srinivasan (2016). Carvone (50 mg/kg b.w.) daily oral administration in diabetic rats for 30 days resulted into a significant decrease in the levels of HbA1c and plasma glucose, while a considerable amelioration in haemoglobin and insulin levels was noted. Carvone administration also caused a reversal in the activities of the carbohydrate metabolic enzymes, enzymatic antioxidants and hepatic marker enzymes that were eventually restored close to normal levels in the diabetic rats. Gliclazide was also used as a standard oral hypoglycaemia drug for comparison. Moreover, carvone treatment was found to reduce the STZ-induced damage in hepatic and pancreas β-cells (Muruganathan and Srinivasan 2016).

The anti-hyperglycaemic property of eugenol was also determined by assessing the activities of key enzymes of glucose metabolism in STZ-induced diabetic rats (40 mg/

kg b.w.) (Srinivasan et al. 2014). Eugenol was intragastrically administered in diabetic rats for 30 days at 2.5, 5 and 10 mg/kg b.w. At an effective dose of 10 mg/kg b.w., remarkable decrease in blood glucose and HbA1c levels and increase in plasma insulin level were reported. Eugenol also caused altered activities of key enzymes involved in carbohydrate metabolism, for instance, pyruvate kinase, glucose-6-phosphate dehydrogenase, hexokinase, fructose-1,6-bisphosphatase, glucose-6-phosphatase and liver marker enzymes (serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and creatine kinase including blood urea nitrogen in serum and blood to significantly reverse to near-normal levels in diabetic rats. In furtherance, eugenol improved body weight and hepatic glycogen level in the treated group, thus demonstrating the potential of eugenol as an anti-hyperglycaemic agent in STZ-induced diabetic rats (Srinivasan et al. 2014).

Likewise results were obtained by myrtenal (Fig. 18.1), a natural monoterpene that has been evaluated for its efficacy as an anti-hyperglycaemic agent as well as its β-cell protective properties in STZ-induced diabetic rats (Rathinam et al. 2014). Myrtenal was orally administered to diabetic rats for 28 days resulting into a significant rise in insulin and haemoglobin levels and reduction in the levels of HbA1c and plasma glucose. The diabetic rats subjected to myrtenal treatment were also protected from loss of body weight. The altered activities of the key metabolic enzymes implicated in carbohydrate metabolism and hepatic enzymes in the diabetic rats were considerably improved by myrtenal administration. Myrtenal also caused an improvement in hepatic and muscle glycogen content in diabetic rats. Further, histopathological analysis unveiled the restoration of reduced islet cells to near-normal conditions, and alteration in the liver architecture was also averted following myrtenal treatment (Rathinam et al. 2014).

The acyclic monoterpene alcohol, geraniol occurring in several medicinal plants exhibits numerous medicinal properties including antidiabetic effect. According to a study by Babukumar et al. (2017), the administration of geraniol at its effective dose of 200 mg/kg b.w. for 45 days relatively enhanced the levels of insulin, haemoglobin and reduced HbA1c and plasma glucose in diabetic-treated rats. Geraniol ameliorated the key enzymes involved in glucose metabolism. Further, the content of hepatic glycogen was improved in diabetic rats that were treated with geraniol. This suggests that geraniol possessed anti-hyperglycaemic potential.

Several EO constituents have also been evidenced to exhibit enhanced antidiabetic effects in combination with antidiabetic drugs. For instance, carvacrol, was evaluated for its anti-hyperglycaemic activity and potential to improve dysregulated carbohydrate metabolism in combination with rosiglitazone in high-fat diet (HFD)-induced type 2 diabetic C57BL/6 J mice (Ezhumalai et al. 2014). The post-oral administration of carvacrol and rosiglitazone was given at 20 mg/kg b.w. and 4 mg/kg b.w., respectively, for 35 days. The HFD mice demonstrated increased levels of insulin, glycosylated haemoglobin and plasma glucose and a declined level of haemoglobin. Besides, there were higher activities of enzymes involved in carbohydrate metabolism in the liver of HFD mice. However, the treatment of diabetic mice with carvacrol and rosiglitazone resulted in increased glucokinase activity due to improved sensitivity of insulin. Likewise, glucose-6-phosphate dehydrogenase

activity was augmented causing enhanced utilization of glucose. In addition, activities of gluconeogenic enzymes (fructose-1,6-bisphosphatase and glucose-6-phosphatase) were significantly reduced in carvacrol- and rosiglitazone-treated mice resulting into reduced levels of blood glucose. Furthermore, the activities of hepatic marker enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase), which were in elevated levels in HFD mice, were restored to normal levels through improved insulin resistance. These results were also supported by histopathological analysis of pancreas, which was in line with the biochemical findings.

18.2.3 Cardioprotective Property

The cardioprotective activities of EOs and their derived components have been widely documented. Included amongst them is 1,8-cineole (Fig. 18.1) a major monoterpenic oxide present in many EOs, which has been evaluated for its effects on systolic blood pressure (SBP) as well as oxidative stress in rats that have been constantly exposed to nicotine via intraperitoneal injection (Moon et al. 2014). A remarkable decline in SBP was observed along with a significant increase in the levels of plasma nitrite in 1,8-cineole-treated rats compared to those exposed to nicotine alone. Besides, a significant rise in the levels of lipid peroxidation and plasma corticosterone was detected in untreated rats. The elevated levels of plasma corticosterone were not reduced by 1,8-cineole; however the increase in lipid peroxidation levels was considerably antagonized in 1,8-cineole-treated rats (0.01 and 0.1 mg/kg). Thus, the results were indicative that the antihypertensive effect of 1,8-cineole led to reduced SBP, which may be linked to the control of nitric oxide levels and oxidative stress in rats that were chronically exposed to nicotine (Moon et al. 2014).

The cardiovascular effects of 1,8-cineole have also been reported by other authors in both in vitro and in vivo experimental models. For instance, Lahlou et al. (2002) demonstrated that 1,8-cineole administration via bolus injections (0.3–10 mg/kg, i.v.) in both conscious and anaesthetized rats induced comparable and dose-dependent declines in mean aortic pressure. Additionally, at the highest dose, i.e., 10 mg/kg, 1,8-cineole caused a significant reduction in heart rate. Moreover, 1,8-cineole-induced hypotension (10 mg/kg) was found to be related to significant bradycardia in both conscious and anaesthetized rats. This resulting effect seemed to be from vagal origin, given that it was significantly decreased with methylatropine pretreatment (i.v.) or by bilateral vagotomy. Furthermore, in the same study, in vitro experiments performed using isolated rat aorta preparations revealed that 1,8-cineole at 0.006–2.6 mM elicited a concentration-dependent decrease in potassium-induced contractions (60 mM). Hence, this study showed that 1,8-cineole displayed hypotensive effects in conscious and anaesthetized rats, which appeared to be associated with active vascular relaxation.

Furthermore, El-Bassossy et al. (2017) showed that geraniol provided effective protection against cardiac dysfunction caused by diabetes. Oral administration of geraniol

(150 mg kg^{-1} day^{-1}) in STZ-injected rats considerably caused the alleviation of the attenuated cardiac systolic function associated with diabetes indicated by hindering the reduction in the rate of rise (dP/dt_{max}) in ventricular pressure and the rise in systolic duration detected in diabetic rats. Additionally, geraniol alleviated impaired diastolic function as demonstrated by restraining the decrease in the rate of fall (dP/dt_{min}) in ventricular pressure and isovolumic relaxation constant (*Tau*) increase in diabetic rats. Additionally, geraniol averted any boost in QTc and T-peak-T-end intervals, left ventricular (LV) ischaemia and arrhythmogenesis markers in diabetic rats. Geraniol also suppressed the exaggerated oxidative stress by preventing 8-isoprotane increase. Moreover, geraniol was able to prevent the inhibition in catalase (CAT) activity although it did not affect the superoxide dismutase (SOD) activity in the heart. Besides, geraniol was observed to partly reduce hyperglycaemia and prevent hypercholesterolemia, but did not have any effect on the adiponectin serum level in diabetic rats.

Moreover, Menezes et al. (2010) investigated the effects of five terpenes, namely, (−)-β-pinene, (+)-α-pinene, (±)-linalool, (±)-citronellol (monoterpenes) and (−)-α-bisabolol (a sesquiterpene) on the blood pressure and heart rate in non-anaesthetized normotensive rats. The monoterpenes were observed to display hypotension associated with tachycardia that could be indicative of an effect on the peripheral vascular resistance with resultant baroreflex response. Alternatively, (−)-α-bisabolol (Fig. 18.1) induced hypotension linked to intense bradycardia, probably as a result of reduced cardiac output. In addition, although all terpenes demonstrated hypotensive effects, terpene hydrocarbons were less efficient as compared to the terpene alcohols.

Linalyl acetate (Fig. 18.1) has also been evaluated for its possible cardiovascular effects on adolescent rats acutely exposed to nicotine (Kim et al. 2017). The levels of nitric oxide, heart rate, systolic blood pressure, vascular contractility and lactate dehydrogenase (LDH) activity were the parameters measured in this study. Significant reductions in both heart rate and LDH activities were noted in linalyl acetate-treated rats. Furthermore, acute nicotine exposure resulted into a minor relaxation effect that was followed by a sustained recontraction stage in contracted mouse aortic rings, while nicotine and linalyl acetate demonstrated a steady relaxation effect. Additionally, treatment with linalyl acetate was found to reduce elevated nitrite levels induced by nicotine. Other authors have also reported the cardiovascular activities of linalyl acetate. For instance, Koto et al. (2006) showed in their study that linalyl acetate as the main component of lavender EO caused vascular smooth muscle relaxation in rabbit carotid arteries, which was partly due to the activation of the nitric oxide/cGMP pathway. Furthermore, linalyl acetate-rich lavender (linalyl acetate 43.73%) aromatherapy was established to cause a rise in coronary flow velocity reserve, hence enhancing coronary circulation in healthy men (Shiina et al. 2008).

Carvacrol and thymol have also been observed to induce vasodilatory effects in isolated rat thoracic aorta preparations (Peixoto-Neves et al. 2010). Both terpenoids caused relaxation of the KCl- and phenylephrine (PHE)-induced contractions of the aortic rings in a concentration-dependent manner. Moreover, carvacrol and thymol were found to totally terminate the phasic component of PHE-provoked

endothelium-containing ring contractions in Ca^{2+}-free medium with 2 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid. In Ca^{2+}-free medium, carvacrol and thymol considerably decreased the $CaCl_2$-induced contractions at 400 μM. In addition, both carvacrol (1000 μM) and thymol (300 μM) significantly diminished the contraction evoked by phorbol dibutyrate (an activator of protein kinase C) at 1 μM. Furthermore, an enhancement was observed in the magnitude of the inhibitory activity in the presence of the thapsigargin, Ca^{2+} pump inhibitor (1 μM). However, none of the two terpenoids were found to modify the resting potential of vascular smooth muscle cells at 1000 μM. Hence, relaxation in rat isolated aorta induced by carvacrol and thymol was endothelium independent, an effect apparently mediated through some mechanisms involving a transduction pathway between release of Ca^{2+} from sarcoplasmic reticulum and by regulating Ca^{2+} sensitivity of the contractile system. It was also proposed that thymol and carvacrol caused blockage of Ca^{2+} influx through the membrane, at low concentrations.

The cardioprotective effects of carvacrol and thymol have also been highlighted by other studies. For instance, Chen et al. (2017) demonstrated in their study that carvacrol provided significant protection of the heart function as well as reduced the myocardial infarct size in myocardial ischaemia/reperfusion (I/R) injured rats. In addition, the levels of SOD and CAT were increased, while the malondialdehyde (MDA) level and cardiomyocytes apoptosis were decreased. Carvacrol treatment also caused the upregulation of phosphorylated ERK without having any effect on p38 mitogen-activated protein kinase (p38MAPK) and c-Jun N-terminal kinase (JNK). Besides, the protective efficiency of carvacrol on cardiomyocytes hypoxic reperfusion (H/R) injury was observed in vitro. Furthermore, carvacrol pretreatment markedly increased the activation of Akt/eNOS pathway in cardiomyocytes subjected to H/R, and carvacrol protective effects were terminated in the presence of the Akt inhibitor LY294002. Hence, the cardioprotective effect of carvacrol was attributable to its anti-apoptotic and antioxidant activities through the activations of the Akt/eNOS and MAPK/ERK signalling pathways. El-Sayed et al. (2016) as well demonstrated that carvacrol and thymol exerted protective effects against doxorubicin (DOX)-induced cardiotoxicity in rats (10 mg/kg i.v.). Administration of carvacrol and thymol (25 and 20 mg/kg p.o., respectively) was found to ameliorate the heart function and oxidative stress parameters. However, thymol was more cardioprotective than carvacrol. A synergistic cardioprotective effect was achieved when carvacrol and thymol were combined together, which might have resulted from its anti-inflammatory including antioxidant and anti-apoptotic activities.

18.2.4 Anticancer Property

In the search for novel anticancer agents derived from plant sources, EOs and their active constituents have been found to be a promising alternative to surmount chemotherapy drug resistance as well as cancer recalcitrance. For instance, thymoquinone (TQ) (Fig. 18.1), a bioactive component obtained from *Nigella sativa* L. seeds and other EOs, has demonstrated notable anticancer potential (Arafa et al. 2011).

Interestingly, TQ was found to significantly restrain doxorubicin-resistant human breast cancer (MCF-7/DOX) cell proliferation. Amongst the different mechanisms proposed, TQ was reported to increase cellular levels of phosphatase and tensin homolog (PTEN) proteins that led to a considerable decline of the phosphorylated Akt cell survival protein. Expression of PTEN was accompanied with increase in PTEN mRNA. Additionally, TQ caused G2/M phase arrest in MCF-7/DOX cells as well as augmented the cellular levels of p53 and p21 proteins. TQ-induced apoptosis was allied with the disruption of mitochondrial membrane potential, caspases activation and cleavage of poly-(ADP-ribose) polymerase-1 (PARP). Upregulation of Bax and downregulation of Bcl2 proteins, resulting in an increase in Bax/Bcl2 ratio, were also observed (Arafa et al. 2011). Mauro et al. (2013) also elucidated the mechanism of action involved in the chemotherapeutic property of D-limonene which was found to be cytotoxic to V79 Chinese hamster cells. It was revealed that D-limonene affected the dividing cells by preventing the assemblage of mitotic spindle microtubules caused by tubulin depolymerization in the early phase of mitosis. Furthermore, both chromosomal segregation and cytokinesis were affected leading to aneuploidy, which consequently resulted into the death of the cell or even genomic instability.

Thymol was also investigated for its chemoprotective effect against genotoxicity induced by bleomycin in human normal lymphocytes as well as anti-proliferative effect on human ovarian cancer cells (SKOV-3) (Arab et al. 2015). Bleomycin (BLM) is an anticancer agent that causes tissue toxicities through DNA damage and death of cells. Samples of blood were treated with BLM following 2 h incubation with thymol at different concentrations (50, 100 and 150 μM). In order to determine the frequency of micronuclei in cytokinesis-blocked binucleated lymphocytes, lymphocytes were cultured with a mitogenic stimulation. A marked decline in the frequency of micronuclei in lymphocytes treated with thymol and BLM was noted compared to blood samples incubated with only BLM (frequency of micronuclei in BLM-treated lymphocytes: 7.79 ± 2.00). Thymol was observed to significantly mitigate micronuclei frequency at doses 50 and 100 μM in BLM-treated lymphocytes (frequency: 2.81 ± 0.99, 2.42 ± 0.16, respectively). Thymol did not demonstrate any genotoxicity in cultured lymphocytes at 150 μM without BLM treatment. Moreover, neither cell protective effect nor improved cell death was observed with thymol pretreatment of SKOV-3 cells. Therefore, this study suggested that thymol provided selective protection to human lymphocytes against DNA damage that was BLM induced without any protection on the killing effect of BLM on cancerous cells (Arab et al. 2015). The effects of thymol on several other cancer lines have been studied as well, whereby their mechanisms responsible for their anticancer or chemopreventive properties have been elucidated (Table 18.1). Likewise, farnesol (Fig. 18.1), an acyclic sesquiterpene alcohol found in the EOs of a variety of plants such as citronella, lemon grass, rose, balsam and neroli (Goossens and Merckx 1997; Azanchi et al. 2014; Krupčík et al. 2015), has been studied for their anticancer effect on different cancer cell lines (Table 18.1).

The anticancer property of patchouli alcohol (PA) (Fig. 18.1), a component isolated from the *Pogostemon cablin* EO, has been tested by Jeong et al. (2013) against

Table 18.1 Effects of thymol and farnesol on various cancer cell lines and anti-cancer mechanisms involved

Cancer type	Cancer cell lines	Anti-cancer mechanisms involved	References
Thymol			
Acute promyelocytic leukaemia	HL-60	Induction of cell cycle arrest at sub-G0/G1 phase, ↑production of reactive oxygen species (ROS) and mitochondrial H_2O_2, depolarization of mitochondrial membrane potential, ↑Bax protein level, ↓expression of Bcl-2 protein, activation of caspase-dependent (caspase-9, -8 and -3 and PARP cleavage) and caspase-independent apoptosis pathways (induction of apoptosis inducing factor (AIF) translocation from mitochondria to cytosol and nucleus)	Deb et al. (2011)
Human colon carcinoma	HCT-116	Induction of oxidative stress through ROS production, DNA and mitochondrial damage, upregulated expression of PARP-1, p-JNK, cytochrome-C and caspase-3 proteins and mitoptotic cell death	Chauhan et al. (2017)
Human glioblastoma	DBTRG-05MG	↑Intracellular Ca^{2+}, release of phospholipase C- and protein kinase C-dependent Ca^{2+} from endoplasmic reticulum, apoptosis and cell death	Hsu et al. (2011)
Human gastric carcinoma	AGS	Cell growth inhibition, apoptosis, production of intracellular ROS, depolarized mitochondrial membrane potential, activation of the proapoptotic mitochondrial proteins (Bax), caspases and PARP in AGS cells	Kang et al. (2016)
Acute T lymphoblastic leukaemia	CEM	Cell cycle arrest at G0/G1 phase	Jaafari et al. (2012)
Mastocytoma	P-815		
Human chronic myelogenous leukaemia	K-562		
Human breast adenocarcinoma	MCF-7		
Human osteosarcoma	MG63	↑ROS, phospholipase C-dependent Ca^{2+} release from endoplasmic reticulum, entry of Ca^{2+} through protein kinase C-sensitive store-operated Ca^{2+} channels, ↑intracellular Ca^{2+}, apoptosis via mitochondrial pathway	Chang et al. (2011)
Farnesol			
Cervical	HeLa	Anti-proliferative, induction of apoptotic cell death, mitochondrial membrane potential loss, downregulated expression of the PI3K and Akt proteins	Wang et al. (2018a)
Lung carcinoma	H460	Induction of endoplasmic reticulum stress and unfolded protein response, activation of p38, ERK, and JNK, induction of apoptosis, activation of caspase-3, -9 and PARP cleavage	Joo et al. (2007)

(continued)

Table 18.1 (continued)

Cancer type	Cancer cell lines	Anti-cancer mechanisms involved	References
Meningiovma	IOMM-Lee	Induction of apoptosis, increase in active caspase-3 and cleaved PARP1, ↓viability	Pfister et al. (2013)
Multiple myeloma	U266	Inhibition of the activation of transcription factor STAT3 (signal transducer and activator of transcription 3) and STAT3-DNA binding activity, inhibition of cell proliferation, ↑accumulation of cells in the sub-G1 phase, induction of apoptotic cell death, activation of caspase-3 and cleavage of PARP, downregulated expression of proteins involved in tumourigenesis including cell cycle regulator protein Cyclin D1 and anti-apoptotic proteins	Lee et al. (2015)
Prostate	DU145	↓Cell proliferation, induction of apoptosis, ↑expression of p53, p-c-Jun N-terminal kinase, cleaved-caspase-3, Bax, and cleaved-caspase-9, ↓expression of p-phosphatidylinositol-3-kinase (PI3K), p-Akt, p-p38, Bcl-2 and p-extracellular signal-regulated protein kinase	Park et al. (2014)

↑Increased, ↓decreased

several cancer lines such as prostate cancer cells (PC3), breast cancer cells (MCF7), human umbilical vein endothelial cells (HUVEC) and pancreatic cancer cells (BxPC3) including human colorectal cancer cells (SW480 and HCT116). PA was noted to exhibit a suppressive activity on cell growth and promote apoptosis dose dependently in the cancer cells. With regard to human colorectal cancer, exposure of PA to SW480 and HCT116 cells caused the increased expression of p21 and downregulated the expression of cell cycle regulatory proteins (cyclin D1 and cyclin-dependent kinase 4 (CDK4)) in a dose-dependent way. Additionally, PA inhibited the expressions of c-myc, HDAC2 (histone deacetylase 2) and HDAC enzyme activity. c-Myc, a transcription factor that mediates progression of cancer, is greatly overexpressed in 60% of colorectal cancer (Smith and Goh 1996), while HDACs are involved in the regulation of cell signalling and gene expression, and their overexpression can result into several diseases including cancers (Haberland et al. 2009). PA also induced activation of the transcriptional activity of NF-κB by increasing the translocation of p65 into the nucleus in human colorectal cancer cells. NF-κB has been reported to cause sensitization of cells to apoptosis as well as promote proapoptotic response (Gibson et al. 2000; Baud and Karin 2009). Moreover, the potent antitumor properties of sesquiterpenes such as β-elemene (Fig. 18.1), has been extensively studied. In this context, it was found to exert anti-glioblastoma effect by restraining cell proliferation and arresting cells in the G0/G1 mediated by the mutually compensatory activation of mitogen-activated protein kinase kinase-3 and -6 (MKK3 and MKK6) (Zhu et al. 2011). Similarly, Liang et al. (2012) showed that β-elemene inhibited viability osteosarcoma cells as a result of apoptosis. However, since treatment with β-elemene also caused upregulation of

hypoxia-inducible factor 1α (HIF-1α) protein inducing partial inhibition of apoptosis, expression of HF-1α was reduced with tiny interfering RNA or co-treatment with an HIF-1α inhibitor (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole). This in turn was observed to greatly improve the antitumor potential of β-elemene. Additionally, more recently Wang et al. (Wang et al. 2018b) demonstrated that β-elemene was able to suppress human cervical cancer SiHa cell proliferation, migration and invasion, as well as promote apoptosis by reducing the Wnt/β-catenin signalling pathway which is associated with tumour formation, invasion and metastasis of different kinds of cancer (Polakis 2000; Hoffmeyer et al. 2012).

EO constituents have also been shown to improve the cytotoxicity of chemotherapy drugs in several cancer cell lines, resulting in the use of reduced concentration of the drug to provide same effect (Legault and Pichette 2007; Rabi and Bishayee 2009). Such synergism has been observed in the case of β-caryophyllene (Fig. 18.1) which, although did not exert any cytotoxic effect alone at the tested concentration, was reported to clearly increase cytotoxicity in combination with paclitaxel in numerous cancer cell lines. It also was pointed out that accumulation of β-caryophyllene in the lipid bilayer resulted into altered permeability that eventually contributed to enhance the permeability of cell membrane for uptake of paclitaxel (Legault and Pichette 2007).

Another such example is a monoterpene alcohol, linalool (Fig. 18.1), which has been tested both individually and combined with doxorubicin, for its effects on two breast cancer adenocarcinoma cell lines (MCF7 WT and MCF7 AdrR). While linalool was found to inhibit cell proliferation only moderately, subtoxic levels of linalool were able potentiate doxorubicin-induced cytotoxic and proapoptotic effects in both cell lines. Strikingly, a considerable synergistic interaction could be observed in MCF7 AdrR (adriamycin-resistant) cells (IC50 of doxorubicin, 16.16 ± 0.94 μM; IC50 of doxorubicin + 50 μM linalool, 1.24 ± 0.26 μM), which could be at least partly attributed to the capacity of linalool to boost doxorubicin buildup and to induce a decline in Bcl-xL levels (Ravizza et al. 2008).

Furthermore, geraniol has been reported to cause sensitization of cancer cells to the 5-fluorouracil, a conventional chemotherapeutic drug, including an increase uptake of the drug (Carnesecchi et al. 2002, 2004). Geraniol also demonstrated to exhibit a chemoprotective action towards rat normal colon cells when a potent carcinogen dimethylhydrazine was administered; this effect was thought to be mediated by reducing DNA damage in contrast with the control where no EO was used (Vieira et al. 2011).

β-Bisabolene (Fig. 18.1), a sesquiterpene present in EO of plants such as *Commiphora guidotti*, was tested for their selective cytotoxicity in both human and murine mammary tumour cells (Yeo et al. 2016). Treatment with β-bisabolene was found to lead to loss of viability of mammary cancer cells as a result of apoptosis. β-Bisabolene was also efficient in decreasing the growth of transplanted 4 T1 mammary tumours in female Balb/C mice in vivo (37.5% decrease). In addition, histological studies performed on the β-bisabolene-treated tumours showed an apparent increase in cell death. Conversely, the number of proliferating cells was decreased in β-bisabolene-treated tumours (Yeo et al. 2016).

18.2.5 Anticonvulsant Activity

Anticonvulsant effects of EOs components have also been reported. As example, Quintans-Júnior et al. (2010) investigated the anticonvulsant effect of three EO components, namely, (-)-borneol, citral and carvacrol in rodents using two animal models of convulsion, i.e., pentylenetetrazole (PTZ)-induced convulsion and maximal electroshock (MES) tests. In their study, the mice were pretreated with the EO components, followed by injection of PTZ at 60 mg/kg after 30 min. Similarly, for MES test, the mice received electroconvulsive shock (130 V, 150 pulses/s, 0.5 s) 30 min after the monoterpenes were injected. The latency for developing convulsions including the percentage protection were then noted. While (-)-borneol and citral were able to stimulate an increase of latency for the development of convulsions by PTZ induction in all doses, carvacrol was effective in only high dose. Besides, these monoterpenes were observed to be efficient in the prevention of tonic convulsions caused by MES. Flumazenil (FLU), an explicit antagonist of the benzodiazepine (BZD) site in the γ-aminobutyric acid ($GABA_A$)-BZD receptor complex (File and Pellow 1986), was employed to examine the role of the participation of $GABA_A$-BZD receptors in the monoterpene-prompted anticonvulsant properties. Interestingly, the presence of FLU was not capable to inverse the anticonvulsant effects of the two monoterpenes, carvacrol and citral, thus implying that there was no direct activation of the BZD site of the $GABA_A$-BZD receptor involved as the mechanism of action. On the contrary, (-)-borneol produced significant antagonistic effect indicating the possible modulation of the GABAergic system via the improvement of $GABA_A$-BZD receptor (Quintans-Júnior et al. 2010).

The effect of isopulegol (a monoterpene alcohol) (Fig. 18.1), in PTZ-encouraged convulsions, was also examined and its possible mechanism of action elucidated (Silva et al. 2009). Intraperitoneal injection of saline, diazepam or isopulegol was administered 30 min before PTZ. The latency for developing convulsions and mortality rate were recorded in mice. Moreover, the antioxidant activity of catalase and the concentrations of lipid peroxidation and reduced glutathione in brain hippocampus were determined. Isopulegol was found to significantly extend mortality and the latency for convulsions in mice in the same way to diazepam. In addition, high dose of isopulegol was observed to induce protection to all animals. FLU was also used in order to investigate on the participation of GABAergic system. The pretreatment of FLU resulted in reduced seizure prolongation latency elicited by both isopulegol and diazepam, even if it was unable to reverse the latency and mortality percentage protection. Also, the monoterpene alcohol significantly averted PTZ-induced rise in lipid peroxidation and maintained normal levels of catalase activity along with the prevention of the PTZ-induced loss of reduced glutathione in the treated mice hippocampus. These findings were indicative that the anticonvulsant and bioprotective activities of isopulegol against convulsions induced by PTZ were probably associated with positive modulation of BZD-sensitive $GABA_A$ receptors together with antioxidative properties.

Terpinen-4-ol (4TRP) (Fig. 18.1), a constituent of EOs obtained from several aromatic plants including *Melaleuca alternifolia* (Carson et al. 2006), was examined for

its anticonvulsant potential by studying the electrophysiological as well as the behavioural activities in rats and mice models (Nóbrega et al. 2014). For this purpose, 4TRP was administered intracerebroventricularly (i.c.v.) (10, 20 and 40 ng/2 μL) and intraperitoneally (i.p.) (25–200 mg/kg) in the animals, while for in vitro experiments, 4TRP was used at 0.1 and 1.0 mM. Based on the results, 4TRP (i.p.) was found to inhibit PTZ-evoked seizures, thus demonstrating anticonvulsant effects. In addition, the protection provided by 4TRP (i.c.v.) against PTZ-induced seizures were found to corroborate with the behavioural results. 3-Mercapto-propionic acid-induced convulsions were used to confirm the involvement of the GABAergic neurotransmission in 4TRP anticonvulsant activity. Moreover, since FLU was not able to reverse the anticonvulsant effect, it could be concluded that 4TRP did not bind to the BZD-binding site although its action was directly or indirectly related to the GABAergic system. Besides, 4TRP inhibited sodium current through voltage-dependent sodium channels and hence its anticonvulsant activity may be associated with changes in neuronal excitability as a result of regulation of these channels (Nóbrega et al. 2014).

As per the study of Sancheti et al. (2014), thymol was found to exhibit potent anticonvulsant as well as antiepileptogenic effects in several experimental models. For instance, the anticonvulsant potential of thymol at a dose of 5–100 mg/kg i.p. was studied using MES-, PTZ-, 4-aminopyridine- (4-AP) and strychnine-induced seizures in rats/mice models. Thymol treatment demonstrated anticonvulsant effect against MES (66.66% death protection at both 50 and 100 mg/kg) and PTZ models (66.66 and 83.33% death protection at 50 and 100 mg/kg, respectively) unlike strychnine and 4-AP models. Thymol capacity to obstruct neuronal voltage-gated Na^+ channels and its positive allosteric modulation of $GABA_A$ receptor could possibly be responsible for thymol anticonvulsant effect in PTZ and MES models (Haeseler et al. 2002; García et al. 2006). Furthermore, thymol was also found to induce marked decrease in locomotor activity (16–80% reduction in locomotion at the doses 25–100 mg/kg, respectively). PTZ-induced kindling model and measurement of MDA and glutathione levels were employed to assess the antiepileptogenic activity of thymol (5–25 mg/kg). Thymol (25 mg/kg, i.p.) was observed to cause significant decrease in the seizure score and MDA level and increase in glutathione level in PTZ-induced kindling animal model (Sancheti et al. 2014).

18.2.6 Antispasmodic Property

EOs and their individual components have also been investigated for their antispasmodic potential. For example, Magalhães and co-workers (1998) showed that *Croton nepetaefolius* EO as well as its components, namely, methyl eugenol (Fig. 18.1), terpineol and cineole, possessed myorelaxant and antispasmodic effects. Both in vivo (mice) and in vitro (guinea pig isolated ileum, cardiac, pyloric and ileocaecal sphincters) models were used to investigate their effects on intestinal motility and mechanical action of intestinal smooth muscle, respectively. The EO was found to be potent modulator of intestinal smooth muscle. Additionally, EO-induced modulation of gastrointestinal smooth muscle activity was indicative

that it was able to assist digestion via its relaxant activities without causing gut stasis. More specifically, the EO was found to increase the intestinal transit of charcoal marker delivered to the mice stomach, while it preferentially reduced basal tonus compared with the amplitude of spontaneous contractions in segments of guinea pig ileum and the sphincters. Similar to the EO, methyleugenol, cineole and terpineol induced concentration-linked relaxation activity of basal tonus and also caused obstruction of 60 mM [K^+]-induced contraction when applied individually (Magalhães et al. 2004). The major compound of *C. nepetaefolius* EO, methyleugenol seemed to play a significant role with better relaxant potency (EC50 for reduction of basal tonus, 9.4 ± 3.3 μg/mL; IC50 for the inhibition of potassium contraction, 12.3 ± 2.7 μg/mL) in comparison with *C. nepetaefolius* EO (EC50, 15.7 ± 4.4 μg/mL; IC50, 18.2 ± 2.3 μg/mL) in isolated intestinal smooth muscle. Cineole (25.37%), the major component found in the EO of *C. nepetaefolius*, comparatively appeared to provide the least contribution (EC50, 322.1 ± 29.8 μg/mL; IC50, 418.9 ± 58.7 μg/mL) as it had only weak relaxant and antispasmodic effect to that of the EO. On the other hand, terpineol by itself induced a higher maximal relaxation of the isolated ileum than the other two constituents (EC50, 70.7 ± 10.7 μg/mL; IC50, 95.4 ± 7.5 μg/mL). However, its presence in the EO did not affect the maximal relaxant response due to its limited amount (4.96%) in the EO, or probably the presence of other constituent(s), which might have inhibited terpineol-induced depressant effect of the basal intestinal tonus.

Likewise, Lima et al. (2000) investigated the effects of methyleugenol (Fig. 18.1) on guinea pig isolated ileum, whereby it was found to reversibly cause relaxation of the basal tonus (EC50: 52.2 ± 18.3 μM), which remained unchanged even by hexamethonium (0.5 mM) or tetrodotoxin (0.5 μM). In addition, relaxation of the ileum pre-contracted with 60 mM KCl was reported. Besides, even though a slight hyperpolarization of the ileum was induced by methyleugenol, it did not have any effect on the depolarized tissues. Moreover, contractions elicited by acetylcholine, KCl and histamine were inhibited by methyleugenol (IC50: 82, 65 and 124 μM, respectively). Thus, it was deduced that methyleugenol caused ileum relaxation by acting directly on the smooth muscle via mechanism mostly independent of membrane potential modifications.

Furthermore, the spasmolytic activity of several monoterpenes present in *Mentha x villosa* leaves EO has been studied by De Sousa et al. (2008). Limonene oxide, pulegone oxide and carvone epoxide, pulegone, (−)-carvone, (+)-carvone, (+)-limonene and rotundifolone were amongst the monoterpenes investigated. Rotundifolone (Fig. 18.1), a component present in many EOs, is known to be spasmolytic. The relationship between the structure and spasmolytic action of rotundifolone and its monoterpene analogues in ileum obtained from guinea pig was examined. With the exception of (+)-limonene and pulegone, all the other analogues were observed to exert spasmolytic effect that was stronger than rotundifolone. The results also revealed that the functional groups and their respective position at the ring of rotundifolone contributed to the ileum relaxation activity and the absence of the oxygenated molecular structure did not really determine the molecule bioactivity. Carvone epoxide (Fig. 18.1) was observed to exhibit significantly stronger spasmolytic behaviour compared to rotundifolone.

Eugenol present in the EOs of many aromatic plants, has also been studied by Leal-Cardoso et al. (2002) for their inhibitory activities on isolated rat ileum. The findings of this study revealed that eugenol was able to cause relaxation of the isolated ileum and inhibit contractions caused by both receptor-independent and -dependent mechanisms. Moreover, eugenol was able to hinder responses of different contractile stimuli, for instance, elevated [K^+] (depolarizing stimulus) and acetylcholine (neurotransmitter) in the absence and presence of a concentration of nifedipine that prevents [K^+]-induced contractions. As a result, a complete relaxation of the tonus increased by 60 mM K^+ with a maximal effect comparable to nifedipine was noted by eugenol. Also, the contraction caused by acetylcholine agonists in solution with nifedipine and Ca^{2+}-free solutions was blocked by eugenol, thus demonstrating its capacity to depress the contraction of the ileal smooth muscle at a certain stage of the signal transduction cascade away from receptors of sarcoplasmic membrane. Therefore, given that the blockage of contractions elicited by 60 mM K^+ and acetylcholine did not cause significant changes to the membrane potential of muscles in normal nutrient solution and complete relaxation of contractions induced by high K^+ was brought about by eugenol without modifying the membrane potential, it was implied that eugenol had a depressant effect on isolated ileum independent of the participation of membrane potential and voltage-dependent Ca^{2+} channels.

The mechanism responsible for the antispasmodic action of menthol (Fig. 18.1), the major constituent in peppermint oil, on the human distal colon has also been evaluated in vitro by Amato et al. (2014). In a concentration-dependent manner, menthol (0.1 mM–30 mM) was found to reduce the amplitude of the spontaneous contractions without having an effect on the frequency and the resting basal tone. Tetrodotoxin (a neural blocker), tetraethylammonium (a blocker of potassium (K+)-channels), 5-benzyloxytryptamine (a transient receptor potential-melastatin8 channel antagonist) or 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (inhibitor of nitric oxide (NO)-sensitive soluble guanylyl cyclase) at concentrations 1 μM, 10 mM, 1 μM and 10 μM, respectively, showed no effect on the inhibitory action of menthol. In contrast, significant reduction was obtained in menthol inhibitory actions in the presence of 3 nM nifedipine (a voltage-activated L-type Ca^{2+} channel blocker). Menthol also decreased the contractile responses resulting from the exogenous application of Ca^{2+} (75–375 μM) in a Ca^{2+}-free solution, or caused by KCl (40 mM) in a concentration-dependent manner. Besides, menthol (1–3 mM) potently reduced both the electrical field stimulation (EFS)-induced atropine-sensitive contractions and contractile responses induced by carbachol. Therefore, menthol was observed to exhibit spasmolytic effects in circular muscle of human colon by the direct inhibition of contractility of the gastrointestinal smooth muscle, via the blockage of Ca^{2+} influx through the sarcolemma L-type Ca^{2+} channels (Amato et al. 2014).

18.2.7 Anti-Inflammatory Property

Various plants and their active components utilized in traditional medicine have been evidenced to be valuable in the treatment of inflammatory disorders (Bernstein et al.

2018; Oguntibeju 2018). Likewise, a series of in vivo, in vitro and in silico studies have been conducted on the anti-inflammatory properties of EOs and their compounds (Pérez et al. 2011; Yadav et al. 2013; Maurya et al. 2014; Andrade 2015; Yang et al. 2016). For instance, Hirota et al. (2010) assessed the anti-inflammatory potential of limonene (Fig. 18.1) obtained from *Citrus junos* Tanaka peel in the treatment of bronchial asthma. For this purpose, the level of monocyte chemoattractant protein-1 (MCP-1), p38 mitogen-activated protein kinase (MAPK), nuclear factor (NF) kappa B as well as the reactive oxygen species (ROS) on human eosinophilic leukaemia HL-60 clone 15 cells was measured. Interestingly, at low concentration (7.34 mM), limonene was found to be effective in hindering the production of ROS for eotaxin-stimulated HL-60 clone 15 cells. Furthermore, a significant reduction was observed in the production of MCP-1 at 14.68 mM of limonene. This was achieved by activating NF-kappa B similar to when the proteasomal inhibitor MG132 was added. Inhibition of cell chemotaxis in a p38 MAPK-dependent manner comparable to the addition of SB203580 (inhibitor of p38 MAPK) was also noted at a concentration of 14.68 mM of limonene.

Two sesquiterpenic constituents, namely, α-humulene and (−)-trans-caryophyllene (Fig. 18.1) isolated from *Cordia verbenacea* EO, were assessed by Fernandes et al. (2007) for their anti-inflammatory properties. Several inflammatory experimental models involving mice and rats and different inflammation inducers were used. For instance, paw oedema caused by carrageenan in mice was strikingly repressed by these two sesquiterpenes (50 mg/kg, oral administration). Besides, both active compounds were found to effectively decrease bradykinin-, ovalbumin- and platelet-activating factor-induced mouse paw oedema. Similarly, the formation of prostaglandin E_2, expression of inducible cyclooxygenase (COX) and nitric oxide synthase caused by carrageenan intraplantar injection in rats were also diminished by α-humulene and (−)-trans-caryophyllene. However, oedema induced by histamine injection was reduced solely by α-humulene. Furthermore, while (−)-trans-caryophyllene was able to reduce only the release of TNFα (tumour necrosis factor-α), α-humulene mostly averted both TNFα and IL-1β (interleukin-1β) production in carrageenan-injected rats upon systemic treatment. The results also revealed that the two sesquiterpenes demonstrated anti-inflammatory activities comparable to those experienced by dexamethasone-treated rats (positive control drug).

Moreover, thymol isolated from EO obtained from the leaves of *Lippia gracilis* (32.68%) is thought to be a chief phytoconstituent accountable for its antinociceptive as well as its anti-inflammatory effects (Mendes et al. 2010). Thymol has been demonstrated to hinder the discharge of arachidonic acid, COX and prostaglandins biosynthesis like prostaglandin E2 in visceral pain model (Mendes et al. 2010). Likewise, thymol at 100 mg/kg was observed to minimize inflammation and encourage wound healing in rodent models by inhibiting leucocytes influx to the wounded regions and as a result prevented oedema (Riella et al. 2012).

Liang et al. (2014) also evaluated the anti-inflammatory property of thymol (10, 20, 40 μg/mL) in lipopolysaccharide (LPS)-stimulated mouse mammary epithelial cells (mMECs). Thymol was seen to significantly hold back the production of IL-6 and TNF-α in LPS-stimulated mMECs. Thymol was also found to suppress the expression of COX-2 and iNOS dose dependently. In addition, the phosphorylation

of NF-κB p65, IκBα, JNK, ERK and p38 mitogen-activated protein kinases (MAPKs) in LPS-stimulated mMECs was blocked by thymol. These findings thus pointed out that thymol anti-inflammatory activity in LPS-stimulated mMECs was brought about by hindering the activation of MAPK and NF-κB signalling pathways (Liang et al. 2014).

Furthermore, Xia et al. (2019) investigated the anti-inflammatory potential of trans-cinnamaldehyde (TCA) (Fig. 18.1), a compound present in *Cinnamomum cassia* Presl on cartilage chondrocytes in vitro and in rat models of osteoarthritis (OA). SW1353 cells and human primary chondrocytes were exposed to varying concentrations of TCA (2–20 μg/mL) for 2 h followed by stimulation of IL-1β, an inflammatory cytokine. TCA showed insignificant effect on cell viability at 10 μg/mL. TCA treatment at concentration 2–10 μg/mL was observed to reduce the levels of expression of matrix metalloproteinases (MMP-1, MMP-3, MMP-13) as well as a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4 and ADAMTS-5) dose dependently in IL-1β-stimulated SW1353 cells. TCA-treated IL-1β-stimulated SW1353 cells at concentration 20 μg/mL also showed reduction in matrix metalloproteinases expression levels, significantly lower than that of 10 μg/mL TCA. Similarly, increase in TCA up to 20 μg/mL also reduced ADAMTS-5, involved in the degradation of cartilaginous matrix, while contrarily ADAMTS-4 showed a significant increase in expression level at 20 μg/mL in IL-1β-stimulated SW1353 cells. Pretreatment of IL-1β-stimulated SW1353 cells with TCA inhibited NF-κB activation, IκBα degradation and increased p-IκBα expression, suggesting that nuclear factor-kappa B (NF-κB) inactivation was caused by TCA. NF-κB activation by stimulation of IL-1β can lead to the expression of proinflammatory mediators thereby causing inflammation (Marcu et al. 2010). Interestingly, TCA was also found to suppress the activation of p-p38 and p-JNK1/2, while p-ERK levels were not considerably affected. IL-1β-Stimulated human primary chondrocytes treated with TCA at 10 μg/mL, considered to be the effective concentration, also demonstrated comparable results to IL-1β-stimulated SW1353 cells. In vivo evaluation to examine the TCA chondrocyte protective effects involved the intraperitoneal injection of TCA (50 mg/kg) in monosodium iodoacetate-induced OA rats. Based on the results, TCA was established to reduce OA progression and the Osteoarthritis Research Society International (OARSI) scores, therefore confirming its cartilage protecting effect and anti-inflammatory property vis-à-vis OA.

18.3 Conclusion and Future Prospects

Plant-derived EOs are complex mixtures of bioactive components responsible for a wide spectrum of biological activities. Several studies have been conducted on EOs, including their individual constituents to evaluate their pharmacological properties. Moreover, studies have been focusing on their underlying mechanisms of actions involved in showing those effects. However, their isolated active compounds, occurring at high amounts have been of particular interests as they are believed to play a major role in the pharmacological actions demonstrated by the EOs.

Nevertheless, it has been reported that EOs minor components can also exert synergistic or antagonistic effects, and as a result contribute to the EOs bioactivities. Therefore, the evaluation of the isolated EO constituents and a better understanding of their corresponding mechanistic actions can aid in more specific and targeted approach in the treatment of several diseases. In addition to acting as a substitute for synthetic drugs with low toxicity, many of the EO constituents have been seen to have drug potentiating effect, resulting into more enhanced effects and concomitantly reducing the concentration of drug being used as well as their possible adverse effects. Thus, this is a very interesting feature that can be further explored by the scientific community given the vast panoply of bioactive components present in volatile oils, and in this way they can be used in combination with synthetic drugs for better treatment and management of diseases.

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Neuroprotective Compounds from Plant Sources and their Modes of Action: An Update

19

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Contents

Abstract

Central nervous system diseases, particularly neurodegenerative maladies like Parkinson's and Alzheimer's diseases are the major public health concerns worldwide, since their prevalence has been increasing, and they are associated with social and financial problems. Owing the limited effectiveness and side effects of pharmacological treatments that are currently available and the fact that several factors are implicated in these diseases, novel treatments acting on multiple molecular targets are required. This chapter focuses on the beneficial effects of plant compounds against neurodegenerative diseases, specifically on

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the main groups of compounds, their sources, and mode of action. Reviewed results showed that there are different plant compounds with the aptitude to target simultaneously several pathological pathways and to affect the activity of numerous enzymes or genes involved in neurological diseases. Nevertheless, the key ability of the reviewed compounds is their capacity to counteract oxidative stress damages and neuronal inflammations. The available results encourage more investigations and clinical trials aimed to develop new treatments for neurodegenerative disorders based on plant compounds.

Keywords
Alkaloids · Antioxidants · Cannabinoids · Flavonoids · Neuroinflammation · Polyphenols

19.1 Introduction

Many disorders of the nervous system are known, and among them epilepsy, stroke, headache disorders, and neurodegenerative diseases are the most prevalent ones. The latter include a heterogeneous group of diseases like Parkinson's (PD), Alzheimer's (AD), and Huntington's diseases (HD) and multiple sclerosis. The pathology of these diseases is complex, progressive, multifactorial, and with many targets and pathways (Hornedo-Ortega et al. 2018; Pohl and Lin 2018). They result in slow neuronal death, which is accompanied by the loss of cognitive functions, and therefore are associated with great social and financial problems. Aging and genetic, lifestyle, and environmental factors are implicated in the pathogenesis of many neurological conditions, and it is expected that their occurrence considerably increases in the coming decade (Peña-Altamira et al. 2017). For instance, it is anticipated that the amount of AD patients increase to 100 million by 2050 (Prince et al. 2013). No cure is currently available for neurodegenerative disorders, and the available treatments merely alleviate the symptoms and delay the disease progression. Moreover, the pharmacological treatments that are available today have limited efficacy and show some side effects. Owing to these limitations and the fact that several factors are implicated in these diseases, a multidimensional approach including lifestyle and dietary interventions seems to the most adequate approach for the management of these diseases (Wang et al. 2018).

Many investigations demonstrated that plant compounds, including dietary phytochemicals possess a multitude of biological features endowing some of these compounds with a great neuroprotective action (Arumugam et al. 2016; Mohanty et al. 2017; Velmurugan et al. 2018; Wang et al. 2019). Indeed, two important compounds currently used in neurodegenerative disease therapy are of natural origin: L-DOPA for PD and galantamine for AD. Phytochemicals can contribute to the control of neurodegenerative disorders not only with their neuroprotective effects, but also by enhancing gastrointestinal function and immunity improvement (Bhullar and Rupasinghe 2013). The correct understanding of plant compounds mechanism of action is fundamental in developing novel neuroprotective agents. Thus, extensive investigations have been

conducted in the last years to study the neuroprotective potentials of plant compounds and to elucidate their mode of actions involved. The purpose of this chapter is to make an outline of the valuable effects of phytocompounds for the management of neurodegenerative diseases and review the main plant compounds with neuroprotective effects, their sources, and mode of action based on the recent data.

19.2 Neurodegenerative Diseases and Main Therapies

Neurodegenerative disorders are more frequent in the elderly population, and their incidence is growing, since the proportion of older people is increasing; thus, they become a threat in this century (Prince et al. 2013; Pohl and Lin 2018). These diseases are caused by the degradation and subsequent loss of neurons, leading to cognitive or functional decline of the patient over time. AD is perhaps the most predominant and overwhelming neurodegenerative disorder, and it is the main reason of institutionalization in the old population (WHO 2017). It causes progressive and irreparable memory deficits, cognitive decline, and even behavior changes. PD is another most frequent of the neurodegenerative diseases related with age, which can also seriously disturb the quality of life. It is a movement disease being the characteristic signs the inactive tremors, bradykinesia, loss of walking and equilibrium, and extrapyramidal rigidity.

The pathogenesis of neurodegenerative diseases is multifactorial with many targets and pathways, although genetic and environmental factors are the most important responsible for their evolution. Oxidative stress associated with accumulation of some aggregated proteins, mitochondrial dysfunction, and neuroinflammation are some of the pathological features involved in these diseases (Farooqui 2012). Dopaminergic treatments, antipsychotic and brain stimulation drugs, and cholinesterase inhibitors are some of the treatments available for their control. In cases of AD, they include the use of exogenous antioxidants, N-methyl-D-aspartate (NMDA) receptor antagonists, and acetylcholinesterase, monoamine oxidase, Aβ, and tau aggregation inhibitors (Sanabria-Castro et al. 2017). After several decades of research, few drugs have been accredited for the management of many neurodegenerative diseases (Newman and Cragg 2012; Cummings et al. 2014). Furthermore, approved drugs merely alleviate the symptoms and delay the disease progression since no cure is currently available for these pathologies. The current research on therapies for neurodegenerative diseases focuses in different potential drug targets.

Mainly, the oxidative stress is the root cause of neurodegenerative disorders (Wang et al. 2018). Oxidative stress involves the disruption of cell redox status, resulting in a disproportion between the generation of reactive oxygen species (ROS) and the antioxidant response. Due to its high oxygen consumption, the brain is the organ more vulnerable to oxidative stress (Yin et al. 2016). Furthermore, the brain is especially disposed to lipid peroxidation owing the high quantity of polyunsaturated fatty acids in neuronal membranes. It is also known that ROS production increases with aging, while endogenous antioxidant defense mechanisms decrease. Additionally, inflammation, protein aggregation, and excessive presence of metal ions, such as iron and copper, can cause oxidative stress, leading to the damage of

biomacromolecules and creating a suitable environment for the pathology and evolution of neurodegenerative illnesses (Kim et al. 2015). The reduction of harmful ROS in the human body but allowing enough ROS to remain in the cell is a promising approach against these diseases. The exogenous supply of antioxidants to counteract the effect of oxidative stress and prevent neuron damage and loss is necessary, if the endogenous antioxidant system is not sufficient. Natural antioxidants, as those present in plants, including plant foods have revealed promising results and hence can be a good alternative to synthetic antioxidants.

Also, chronic inflammations play a vital role in the initiation and evolution of neurodegenerative disorders. Neuroinflammatory processes are implicated in successive events causing neuronal injury. During the inflammatory process, nuclear factor-kappa B (NF-κB) and inflammatory cytokines, like tumor necrosis factor (TNF)-α, IL-6, and IL-1β, are activated (Wang et al. 2018). Activated microglia in the brain releases unnecessary and detrimental ROS and reactive nitrogen species (RNS). Oxidative stress and neuroinflammation in association are both involved in neurodegenerative disorders. Furthermore, mitochondrial dysfunction is boosted by oxidative stress and neuroinflammation mediated by microglia. In fact, oxidative stress, neuronal inflammation, and mitochondrial impairment are interconnected in neuronal damage (Wang et al. 2018; Zheng et al. 2018a).

The search for effective tactics for neurodegenerative diseases using phytochemicals has augmented in the last years, due to the inefficacy and side effects of currently available therapies based on the use of synthetic compounds. Particularly, the supplement of dietary phytochemicals has demonstrated to be a hopeful nutritional intervention for the management of these disorders (Hornedo-Ortega et al. 2018). This is supported by many investigations, including preclinical trials that have demonstrated the neuroprotective effects of some phytochemicals mediated via different mechanisms, such as antioxidant, anti-inflammatory, anticholinesterase, anti-tauopathy, antiamyloidogenic, antiapoptotic, and neurotrophic effects (Fig. 19.1) (Velmurugan et al. 2018).

19.3 Main Sources of Plant Neuroprotective Compounds

Many studies have indicated that the risk of incidences of some diseases, including neurodegenerative ones depends on the dietary pattern followed by individuals. Many studies demonstrated that a broad range of phytochemicals can counteract the effects of oxidative stress and the neuronal inflammation process (Hornedo-Ortega et al. 2018; Velmurugan et al. 2018). Nutritional interventions, including a vast range of plant products are considered an effective approach against neurological conditions, particularly due to their anti-inflammatory and antioxidant capabilities (Wang et al. 2018) and their capacity to improve gastrointestinal and immunological functions (Bhullar and Rupasinghe 2013). Thus, diet intervention is beneficial for the ageing process, maintaining physical and cognitive health (Wang et al. 2018) and preventing and/or delaying the progression of neurological diseases (Hornedo-Ortega et al. 2018). Fruits and vegetables as well as aromatic herbs and spices are the main sources of antioxidants as they possess several neuroprotective

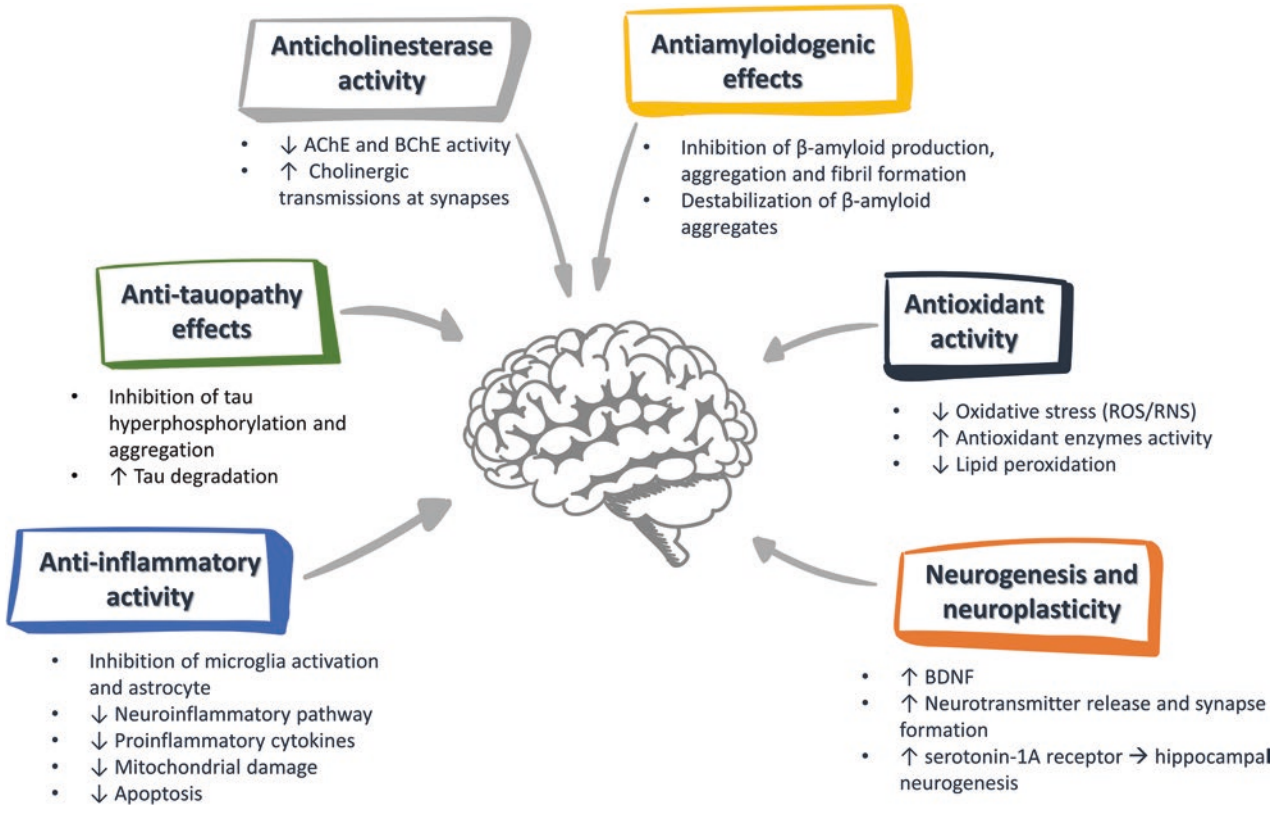

Fig. 19.1 Some of the multiple biological features of plant compounds contributing to their neuroprotective properties. *AChE* acetylcholinesterase, *BChE* butyrylcholinesterase, *BDNF* brain-derived neurotrophic factor, *ROS* reactive oxygen species, *RNS* reactive nitrogen species

phytochemicals. There are increasing evidences about the beneficial effects of Mediterranean diet against age-related pathological conditions (Hornedo-Ortega et al. 2018). This diet pattern is linked with a decrease in cognitive failure and dementia prevalence. The beneficial effects of this dietary pattern are associated with the high consumption of plant foods and plant-derived products as fruits, vegetables, aromatic herbs, olive oil, and adequate quantities of red wine that are all recognized sources of bioactive phytochemicals (Hornedo-Ortega et al. 2018). The protective role of food polyphenols in preventing neurodegenerative ailments is particularly well-documented and is mainly due to their strong free radicals scavenging capacity (Omar et al. 2017; Losada-Barreiro and Bravo-Díaz 2017). Fruits and vegetables contain several phenolic compounds, mainly high amounts of flavonoids, are well recognized to have antioxidant and anti-inflammatory activities (Spagnuolo et al. 2018). Among fruits, berries are probably the richest sources of neuroprotective phenolics (Kelly et al. 2018), and cruciferous are some of the vegetables recognized for their neuroprotective properties. Wine and olive oil are two of the main sources of important neuroprotective polyphenols among Mediterranean diet products. Olive oil is rich in hydroxytyrosol and oleuropein, as well as other bioactive phenolics (Casamenti and Stefani 2017), and red wine is rich in resveratrol, one of the most recognized neuroprotective compounds (Colica et al. 2018).

Herbs and spices used for food, cosmetic, and medicinal purposes are also rich sources of antioxidants (Embuscado 2015). They contain a great diversity of bioactive compounds, from which phenolics and essential oils are the most important. Among the great diversity of plants, Mediterranean species mainly from the Apiaceae and Lamiaceae families (e.g., *Coriandrum sativum* L., *Origanum vulgare* L., *Salvia* sp., *Mentha* sp., *Rosmarinus officinalis* L., *Thymus* sp.) are some of the most relevant ones (Benny and Thomas 2019). Essential oils from these plants inhaled or orally administered cross the blood-brain barrier and exert their psychological properties at the brain level, having beneficial effects in several neurological conditions (Benny and Thomas 2019). Data from many studies indicated that aromatic plant compounds including essential oil components and phenolics affect different pathological targets involved in neurodegenerative disorders (e.g., oxidative stress, Aβ deposition, neurofibrillary tangles, cholinergic hypofunction, glutamatergic abnormalities) (Ayaz et al. 2017). In addition to dietary plants, some well-known medicinal herbs are also important sources of neuroprotective compounds; some important examples are *Ginkgo biloba* L., *Panax ginseng* Meyer, and *Cannabis sativa* L.

19.4 Main Groups of Plant Compounds with Neuroprotective Effects

The most important phytochemicals with recognized preventive and/or therapeutic properties against neurodegenerative disorders belong to the group of polyphenols, isothiocyanates, alkaloids, and cannabinoids (Table 19.1). In addition to these four groups, there are some other chemical compounds with a remarkable action at the

Table 19.1 Main neuropharmacological properties and source(s) of selected plant compounds with neuroprotective effects

Classes	Subclasses	Compound	Neuropharmacological properties	Main source(s)	Reference(s)
Polyphenols	Flavonoid (Flavanol)	Catechin	Butyrylcholinesterase (BChE) inhibitor, ↓ β-amyloid accumulation	Fruits, green tea, leafy green or root vegetables, broad beans, and green beans	Omar et al. (2017), Hajialyani et al. (2019)
	Flavonoid (Flavanol)	Epigallocatechin-3-gallate	Antioxidant, anti-inflammatory, and antiapoptotic properties; ↓ β-amyloid accumulation; acetylcholinesterase (AChE) inhibitor; ↑ ATP levels in mitochondria; ↑ cognitive impairment	Green tea, berries, grapes, cocoa	Libro et al. (2016), Reglodi et al. (2017), Velmurugan et al. (2018), Manzinea et al. (2019)
	Flavonoid (Flavanone)	Hesperidin	Anti-inflammatory, antioxidant, and antiapoptotic properties; ↓ β-amyloid accumulation; AChE and lipid peroxidation inhibitor; ↑ catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD) activities; corrects Aβ-induced mitochondrial abnormalities; ↓ depression symptoms in Parkinson's disease (PD); ↑ locomotion efficiency, learning and memory function in patients with Alzheimer's disease (AD)	*Citrus* spp., grapefruits	Hajialyani et al. (2019)
	Flavonoid (Flavonol)	Quercetin	Antioxidant, anti-proliferative, and antiapoptotic properties; ↑ GSH and SOD activities; prevents cytotoxicity induced by H_2O_2; ↑ biogenesis of mitochondria; ↓ motor deficits	Fruits, vegetables, tea, nuts, *Ginkgo biloba* L., *Hypericum perforatum* L., *Sambucus canadensis* L.	Li et al. (2016), Reglodi et al. (2017), Velmurugan et al. (2018), Hajialyani et al. (2019)

(continued)

Table 19.1 (continued)

Classes	Subclasses	Compound	Neuropharmacological properties	Main source(s)	Reference(s)
	Non-flavonoid (Curcuminoid)	Curcumin	Antioxidant and anti-inflammatory properties; prevents maturation of amyloid-β precursor protein (APP); reverses progression of tau/amyloid pathology in AD; helps in regeneration of neurons; ↑ brain-derived neurotrophic factor (BDNF) for maintenance of neurons in central nervous system (CNS); ↑ dopamine, norepinephrine, and 5-HT levels in CNS; ↑ cognitive functions; ↓ motor deficits	*Curcuma longa* L.	Reglodi et al. (2017), Wasik and Antkiewicz-Michaluk (2017), Maiti and Dunbar (2018), Velmurugan et al. (2018), Wang et al. (2018), Khazdair et al. (2019), Hatami et al. (2019), Manzinea et al. (2019)
	Non-flavonoid (phenolic acid, hydroxycinnamic acid)	Rosmarinic acid	Antioxidant and anti-inflammatory properties; AChE and BChE inhibitor; ↓ mitochondrial dysfunction and reactive oxygen species production; ↓ neuronal excitability; ↑ cholinergic tone	Lamiaceae species	Omar et al. (2017), Fachel et al. (2019)
	Non-flavonoid (Stilbene)	Resveratrol	Anti-inflammatory, antioxidant, and antiapoptotic properties; ↑ BDNF levels in the hippocampus; suppresses activation of glial cells; ↓ β-amyloid accumulation; prevents neurotoxicity in PD; ↑ SOD and CAT activities; ↓ malondialdehyde (MDA); normalizes mitochondrial function; ↓ anxiety and cognitive deficits; improves spatial learning and memory; activation of sirtuin 1	Grapes, berries, red wine, blueberries, peanuts, tea	Libro et al. (2016), Reglodi et al. (2017), Wasik and Antkiewicz-Michaluk (2017), Andrade et al. (2018), Colica et al. (2018), Velmurugan et al. (2018), Wang et al. (2018)

Alkaloids	Indole alkaloid	Isorhynchophylline	Antioxidant and antiapoptotic properties; ↓ β-amyloid accumulation; positive effects against dementia, amnesia, ischemia and epilepsy	*Uncaria rhynchophylla* (Miq.) jacks.	Hussain et al. (2018)
	Isoquinoline alkaloid	Berberine	Anti-inflammatory, antioxidant, and antiapoptotic properties; ↓ β-amyloid accumulation; AChE, BChE, and monoamine oxidase inhibitor; regulates neurotrophin levels; protects neuronal cells from neurotoxicity; improves learning deficits and long-term spatial memory; positive effects against depression and amnesia	*Hydrastis canadensis* L., *Coptis chinensis* Franch., *Berberis* spp.	Libro et al. (2016), Hussain et al. (2018), Lin and Zhang (2018), Velmurugan et al. (2018), Fan et al. (2019), Yuan et al. (2019)
	Isoquinoline alkaloid	Galantamine	Antioxidant properties; AChE inhibitor; ↓ β-amyloid accumulation; ↑ cognition, memory, and sleep quality; positive stimulation in hippocampal neurogenesis	Amaryllidaceae species	Libro et al. (2016), Hussain et al. (2018)
	Isoquinoline alkaloid	Morphine	Antioxidant properties; ↓ β-amyloid accumulation; ↑ GABA levels in synapse of brain; ↓ agitation behaviors and depression	*Papaver somniferum* L.	Libro et al. (2016), Hussain et al. (2018)
	Lycopodium alkaloid	Huperzine A	Anti-inflammatory, antioxidant, and antiapoptotic properties; AChE inhibitor; ↓ β-amyloid accumulation; improves mitochondrial energy metabolism and memory deficits	*Huperzia serrata* (Thunb.) Trevis.	Libro et al. (2016), Hussain et al. (2018)

(continued)

Table 19.1 (continued)

Classes	Subclasses	Compound	Neuropharmacological properties	Main source(s)	Reference(s)
	Methylxanthine derivative	Caffeine	Antioxidant and anti-inflammatory properties; ↓ neurotoxicity; ↓ β-amyloid accumulation and tau phosphorylation; ↑ cortical activity, metabolism of cerebral energy, and extracellular levels of acetylcholine; ↓ risk of dementia and memory decline	*Coffea arabica* L.	Libro et al. (2016), Hussain et al. (2018), Pohl and Lin (2018)
	Piperidine alkaloid	Piperine	Anti-inflammatory and antioxidant properties; AChE inhibitor; ↑ neuronal density in hippocampus in low concentrations; modulates the neurotransmitter systems in epilepsy; anticonvulsive agent	*Piper* spp.	Hussain et al. (2018)
	Pyridine alkaloid	Nicotine	Antioxidant, anti-inflammatory, and antiapoptotic properties; neuroprotective effect against Aβ toxicity; ↑ memory performance	*Nicotiana tobaccum* L.	Libro et al. (2016), Hussain et al. (2018)
	Pyrroloindole alkaloid	Physostigmine	AChE and BChE inhibitor, cognitive enhancer	*Physostigma venenosum* Balf.	Omar et al. (2017), Hussain et al. (2018)
Isothiocyanates		6-MSITC	Antioxidant, anti-inflammatory, and antiapoptotic properties; ↑ GSH activity; neuroprotective effects in PD; ameliorates Aβ-induced memory impairments	*Wasabia japonica* (Miq.) Matsum. (wasabi)	Sita et al. (2016), Morroni et al. (2018)
		Moringin	Antioxidant and anti-inflammatory properties; ↓ activity of MDA and AChE; ↑ SOD and CAT activities; ↑ cholinergic function; ↑ spatial memory	*Moringa oleifera* lam. (drumstick tree)	Giacoppo et al. (2015), Libro et al. (2016)

		Sulforaphane	Antioxidant and anti-inflammatory properties; ↓ β-amyloid accumulation and peroxidation in AD; AChE inhibitor; protects against rotenone-induced neurotoxicity in PD; ↑ proteasomal and autophagic activities in Huntington's disease; ↓ microglia activation and ↑ inflammatory markers	Brassica vegetable	Giacoppo et al. (2015), Libro et al. (2016), Sita et al. (2016), Pohl and Lin (2018)
Cannabinoids	Phytocannabinoid	Cannabidiol	Antioxidant, anti-inflammatory, and anti apoptotic properties; ↓ β-amyloid accumulation and tau phosphorylation; ↑ neurogenesis; regulates microglial cell migration; prevents the development of cognitive deficits in AD	*Cannabis sativa* L.; *Cannabis indica* lam.	Omar et al. (2017), Morales et al. (2017b), Watt and Karl (2017)
	Phytocannabinoid	Delta-9-tetrahydrocannabinol	Anti-inflammatory properties, mediates neuroprotection through PPARγ-dependent restoration of the mitochondrial material	*Cannabis sativa* L.	Maurya and Velmurugan (2018)

central nervous system, for instance, ginsenosides, terpenoids from essential oils, and polysaccharides (Aazza et al. 2011; Zheng et al. 2018b; Gao et al. 2018).

Polyphenols are probably the main and most investigated class of natural composites and are mostly present in vegetables and fruits, as well as in aromatic and medicinal plants. They include about 8000 structures and can be divided in non-flavonoids and flavonoids (Fig. 19.2) (Manach et al. 2004). Non-flavonoid chemical constituents comprise phenolic acids, stilbenes, lignans, and other compounds as displayed in Fig. 19.2. Flavonoids include about 4000 compounds and are divided in flavanols, flavones, flavonols, isoflavones, flavanones, and anthocyanins. Epidemiological studies indicated that intake of phenolic-rich products can be linked with a decreased risk of chronic diseases as is the case of neurodegenerative disorders (Renaud and Martinoli 2019). The biological properties of polyphenols are related to their structural characteristics, which include the presence of phenol rings in the molecule, variable hydroxylation patterns and conjugated double bonds. Polyphenols prevent and obviate neurodegenerative disorders by different mechanisms, e.g., by reducing oxidative stress, affecting amyloid aggregation, inhibiting enzyme activity, and reducing inflammation by regulating signaling pathways and cytokine expression (Table 19.1) (Renaud and Martinoli 2019).

The neuroprotective effect of flavonoids, the most abundant group of polyphenols in foods, has been frequently described, and is mainly associated with the antioxidant and anti-inflammatory features of these compounds (Spencer et al. 2012; Singh et al. 2017; Spagnuolo et al. 2018). The consumption of flavonoid-rich products is linked with the inhibition of biochemical processes involved in brain aging and in the prevention of neurodegenerative disease development (Spencer et al. 2012). The structure of flavonoids has in common a 15-carbon skeleton structured in 2 aromatic rings intertwined by another carbon chain. The antioxidant effects of these compounds are related to their ability to scavenge oxidants and free radicals (Singh et al. 2017) and reduce neuroinflammation by regulating microglial cells, particularly by modulating mitogen-activated protein kinases (MAPKs) and NF-κB signaling pathways (Spencer et al. 2012; Spagnuolo et al. 2018). Catechin, quercetin, hesperidin, and epigallocatechin-3-gallate (EGCG) present in tea, cocoa, and different fruits (mainly berries and citrus) and vegetables are some of the dietary flavonoids showing important neuroprotective properties (Table 19.1). Among flavonoids, EGCG is widely found in green tea and is one of the most reported compounds with neuroprotective properties. Recent studies suggest that green tea consumption decreases the occurrence of neurodegenerative diseases (Pervin et al. 2018). EGCG is the only polyphenolic compound that has reached the phase III of clinical testing (Levin et al. 2016).

Isothiocyanates are sulfur-containing compounds derived from myrosinase hydrolysis of glucosinolates and are mainly present in vegetables of the family *Brassicaceae*, although they can also occur in *Moringaceae* plants. Several studies indicated that regular consumption of *Brassicaceae* vegetables has positive effects in preventing certain chronic diseases including neurodegenerative diseases. These compounds can modulate oxidative stress and inflammatory processes interacting with the nuclear factor erythroid 2-related factor 2 (NrF2) pathway and therefore are

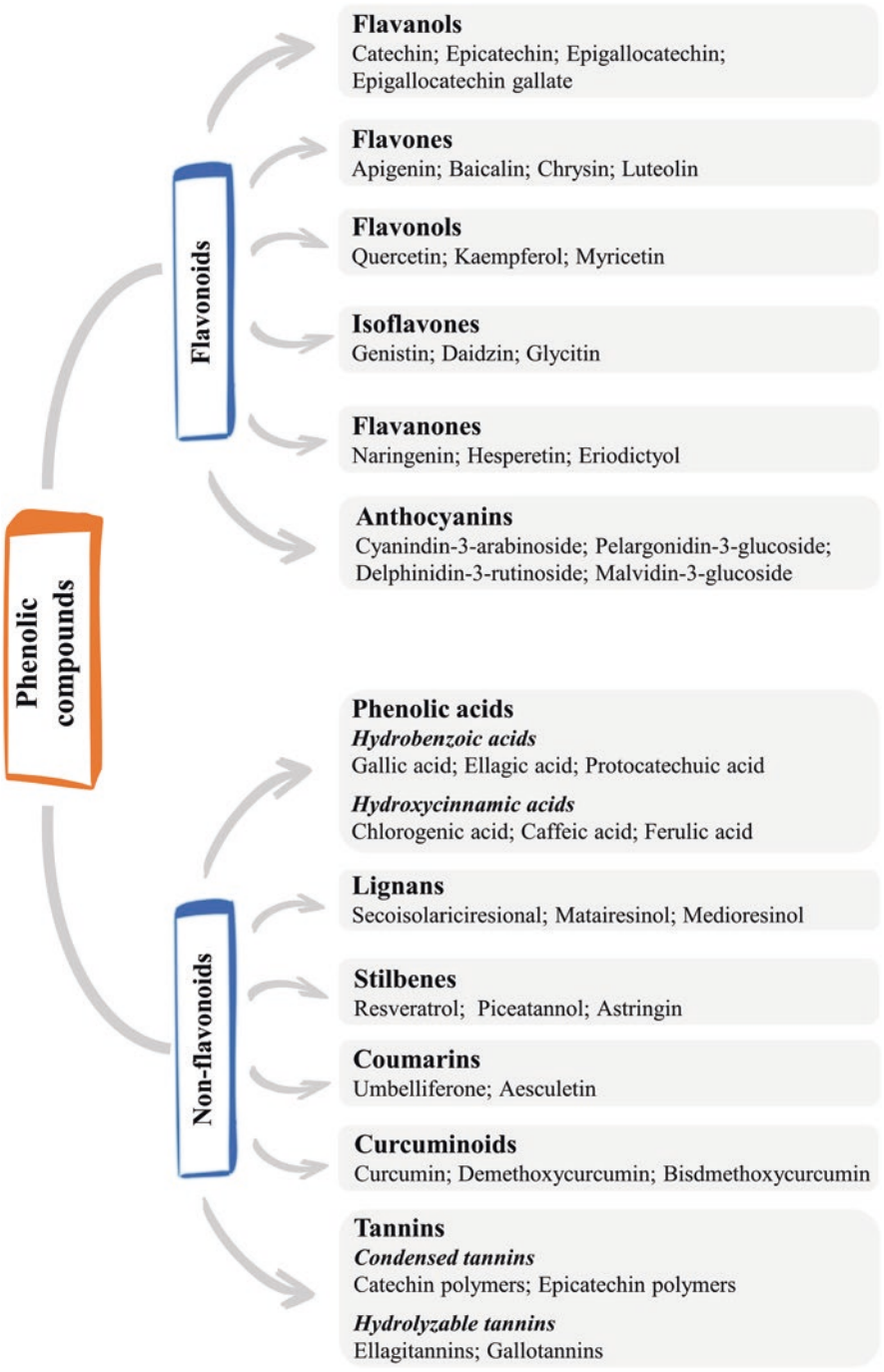

Fig. 19.2 Classification of polyphenols and some examples of each group. Based on Manach et al. (2004)

promising compounds in the deterrence and treatment of cognitive diseases, particularly AD (Giacoppo et al. 2015; Sita et al. 2016). Some important compounds of this class are isothiocyanate, sulforaphane, erucin, moringin, phenethyl isothiocyanate, and 6-(methylsulfinyl)hexyl.

Alkaloids are naturally found in several plant species, but plants of S*olanaceae*, *Papaveraceae*, *Ranunculaceae*, and *Amaryllidaceae* are a rich source of these compounds. These phytocompounds have showed a wide range of biological properties and are separated into several groups depending on their natural sources, pharmacokinetics, and chemodiversity. The role of these compounds in dementia has been expansively explored, and two alkaloid-based drugs, namely, rivastigmine and galantamine, were approved by the Food and Drug Administration (FDA, USA) for the treatment of AD, renewing the attention in alkaloids for dementia treatment. Alkaloids exert their beneficial effects against neurodegenerative diseases by several mechanisms as depicted in Table 19.1. In addition to their capacity to modulate the neurotransmitter system, alkaloids have antiamyloidogenic, antioxidant, anti-inflammatory, antidepressive, and anticonvulsive properties (Hussain et al. 2018). Galantamine, rivastigmine, berberine, morphine, physostigmine, piperine, and caffeine are some of the most important alkaloids among many bioactive compounds within this group.

Cannabinoids are a group of lipid-soluble compounds, present particularly in the plant, *Cannabis sativa* L., and have long been utilized for therapeutic purposes. The use of these compounds in human health increased exponentially in recent years, and the investigation in these compounds is focused on their neuroprotective potential and also on their capacity in attenuating AD-related symptoms. The main advantage of these compounds as neuroprotective agents is their broad-spectrum profile and activity at several molecular sites including within and outside the endocannabinoid system. By combining a broad range of effects, these compounds can regulate neuronal homeostasis and survival (Fernández-Ruiz et al. 2015). Delta-9-tetrahydrocannabinol (Δ^9–THC) is the most investigated compound; however, its psychotropic character limitits therapeutic usage. Cannabidiol, a non-psychotropic cannabinoid has been recently investigated for its effects in neurodegenerative diseases, showing promising results both in in vitro and in vivo models of dementia. Its neuroprotective properties are described comprehensively in the next section.

19.5 Neuroprotective Effects of Selected Plants/Plant Compounds

In this section, some plant species and their compounds showing remarkable neuroprotective properties are addressed in more detail.

19.5.1 *Ginkgo biloba*

Leaves and seeds of the Chinese tree, *Ginkgo biloba* L., belonging to Ginkgoaceae family have been used for centuries in the Chinese traditional medication for the treatment of different neurological conditions. Presently, standardized extracts from this plant are also used, principally in Germany and United States, mainly as a cognitive enhancer (Singh et al. 2017; Wąsik and Antkiewicz-Michaluk 2017). Four standardized extracts have been used in clinical trials, for instance, EGb-761 (*G. biloba* extract, Ginkor) contains mainly flavonol glycosides, such as kaempferol, isorhamnetin, and quercetin, ginkgoin acids, ginkgolin acids, and terpenes like bilobalides and ginkgolides A, B, C, and M (Singh et al. 2017). The antioxidant and neuroprotective properties of this extract have long been recognized. Different in vitro and in vivo investigations showed that this extract exerts its antioxidant effects through different mechanisms, such as free radicals scavenging activity, inhibition of ROS production, activity of antioxidant enzymes, and p-regulation of protein level (Wąsik and Antkiewicz-Michaluk 2017). Some studies also indicated that the mechanism involved in the neuroprotective properties of EGb-761 is related to its capability to inhibit monoamine oxidases A and B activity and to prevent the conversion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to 1-methyl-4-phenylpyridinium (MPP^+) ion (Sloley et al. 2000; Rojas et al. 2004). The compounds present in this extract can cross the blood-brain barrier and therefore induce their effects in the central nervous system (De Feudis and Drieu 2000). The available data demonstrated that the antioxidant and anti-inflammatory features of *G. biloba* standardized extract contribute to restore brain homeostasis in patients with anxiety, dementia or other neurodegenerative disorders, although more consistent clinical trials are still encouraged. The results available indicated that the extract from this plant is relatively safe even when consumed in parallel with other drugs (Singh et al. 2017).

19.5.2 *Panax ginseng*

Panax ginseng Meyer. is a medicinal plant, native to Korea and China and also cultivated in the eastern Asian countries. This plant roots have been employed in the folk medicines to treat several ailments. The beneficial effects of this plant are related to its several active components, particularly the ginseng saponins ginsenosides and also phenolic compounds, polyacetylenes, alkaloids, polysaccharides, sesquiterpenes, and oligopeptides (Kim et al. 2018a, b). This plant showed several biological properties like its capacity to improve the blood circulation, immune system, and memory as well as its antioxidant and other properties (Kim et al. 2018a, b). The activities of *P. ginseng* in preventing neurodegenerative disorders are mainly associated with the antioxidant and immunomodulatory properties of ginsenosides. The basic structure of these compounds is similar, i.e., they comprise 30 carbon atoms and are organized in four rings of steroid nuclei (Guo et al. 2014). As recently reviewed by Zheng et al. (2018a, b), ginsenosides have protective and therapeutic

effects on neurological disorders. These compounds exhibit anti-inflammatory action by interfering with various signaling pathways and antioxidant and anti-aging properties and have beneficial effects on depression, epilepsy, cerebral ischemia reperfusion injury, AD and PD (Zheng et al. 2018a, b). There are several research works on the beneficial effects of *Panax* extracts and its components in animal models of neurodegenerative diseases, mainly in AD and PD as reviewed by different authors (Ong et al. 2015; Kim et al. 2018a, b). For instance, *P. ginseng* extracts showed neuroprotective effects in rat models by counteracting the progressive glycation end product-induced memory loss and downregulating the receptor for advanced glycation end products (RAGE)/NF-kB pathway (Tan et al. 2015). *P. ginseng*, in particular ginsenosides, act not only as antioxidants and free iron scavengers but also as modulators of metabolism, intracellular neuronal signaling, both cell survival and cell death genes, and mitochondrial functions (Kim et al. 2018a). In a recent review, Kim et al. (2018b) concluded that the combination of ginseng compounds with conventional drugs used in AD therapy is more advantageous in the management of this disease than the monotherapy.

Many investigations proved the beneficial effects of *P. ginseng* and its compounds in controlling neurodegenerative disorders, and clinical results indicate no serious adverse effects, although it can modify anticoagulation with warfarin and blood hemostasis. However, data available is still inconclusive and more well-designed clinical studies are required.

19.5.3 Resveratrol

Resveratrol (3,5,4-trihydroxy-trans-stilbene) (Fig. 19.3a) is a stilbene (non-flavonoid polyphenol) that is the mainly found in red grapes and wine, although it is also found in other plants, namely, berries, peanuts, green tea, black tea, etc. This

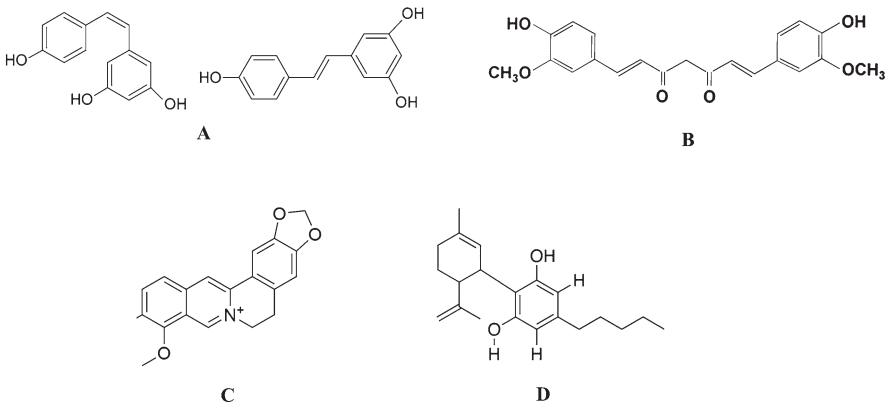

Fig. 19.3 Chemical structure of some important plant compounds with neuroprotective effects: *cis*- and *trans*-resveratrol (**a**) curcumin (**b**), berberine (**c**), and cannabidiol (**d**)

compound is a phytoalexin produced by plants as a response to several biotic and abiotic stimuli as fungal infections and UV radiation (Li et al. 2012). Some investigations showed that this compound has a positive effect on human health including on neurological conditions (Rauf et al. 2017). Indeed, this is one of the most investigated plant compounds concerning the neuroprotective properties. In addition to the properties related to the central nervous system and anti-aging, antioxidant, anti-inflammatory, and neuroprotective effects, this compound also shows several other biological properties including antiapoptotic, cardioprotective, antitumor, and antidiabetes.

This compound exerts its neuroprotective effects through different mechanisms (Table 19.1) but is mainly associated with the protection of neurons from oxidative damage and toxicity effects and to the deterrence of apoptotic neuronal death (Andrade et al. 2018). The antioxidant ability of resveratrol has also been extensively described (Colica et al. 2018) and is related to structural characteristics of the molecule, i.e., three hydroxyl groups attached to the aromatic rings confer the capacity to capture free radicals (Rege et al. 2014). Antioxidant capacity of this compound has been also attributed to its capacity to stimulate the expression of endogenous antioxidant enzymes as superoxide dismutase and catalase, decreasing the malondialdehyde content in mouse brain, reducing copper-catalyzed oxidation, and inhibiting lipid peroxidation (Mokni et al. 2007). Anti-inflammatory effects of resveratrol are mainly related to its capacity to inhibit cyclooxygenases and 5-lipoxygenase activities and consequently the suppression of prostaglandins, thromboxanes, and leukotriene (Kutil et al. 2014), to attenuate pro-inflammatory factors like platelet-activating factor (PAF), tumor necrosis factor (TNF), and histamine (Wiciński et al. 2018). The neuroprotective properties of resveratrol are also associated with its capacity to activate deacetylase enzymes like sirtuin 1 (SIRT1) (Sarubbo et al. 2018).

Although this compound has revealed promising properties against neurological diseases and is able to cross the blood-brain barrier, its pharmacokinetic properties limitits therapeutic applications. It is extensively metabolized and rapidly eliminated, showing a poor bioavailability and a great instability, in spite of its lipophilic nature. Thus, several investigations have been conducted concerning the use of several strategies to improve the pharmacokinetic characteristics, such as its encapsulation for delivery in the brain (Ethemoglu et al. 2017; Trotta et al. 2018). As recently reviewed by Chimento et al. (2019), these strategies showed promising results vitro and in vivo, but their efficacy must be investigated in preclinical and clinical studies.

19.5.4 Curcumin

Curcumin (Fig. 19.3b), 1,7-bis[4-hydroxy, 3-methoxy phenyl]-1,6-hepta-diene-3,5-dione, is a non-flavonoid polyphenol. This yellow pigment is the main component of curry spice (*Curcuma longa* L.) roots, which is a commonly used spice in Asian. Curry consumption has long been associated with cognitive function improvements

in the elderly. Indeed, some studies indicated that incidence of AD in India is lower than in the US population. Curcumin showed several biological effects including anti-inflammatory, antidiabetic, antioxidant, anticancer, and neuroprotective properties. The antioxidant, anti-inflammatory, and antiamyloidogenic effects of this compound, among other (Table 19.1), make it a promising candidate for neurological diseases therapy (Maiti and Dunbar 2018; Hatami et al. 2019). The antioxidant properties of this compound were confirmed in different in vitro and animal models and are mainly related to the presence of a phenolic group linked with two methoxy groups in its structure. This compound revealed radicals scavenging and metal-chelating capacities, improves brain antioxidant status, and protects cells from lipid peroxidation (Maiti and Dunbar 2018). It can attenuate the development and progression of neuroinflammatory disorders through different mechanisms as reducing inflammatory mediators (e.g., TNF-alpha, IL-1 beta, nitric, NF-kappa B gene expression, inhibition of cyclooxygenase 2, and NOS), affecting mitochondria dynamics and epigenetic changes (Hatami et al. 2019). Also, it has the aptitude to bind with amyloid plaques by inhibiting NF-κβ, thus diminishing AD pathogenesis. Research has revealed that curcumin derivatives have more lipophilic properties and higher capability to cross the blood-brain barrier and more affinity for amyloid plaques. Also, its association with lipophilic compounds was shown to improve its bioavailability (Mourtas et al. 2011). Nevertheless, well-ordered and randomized clinical trials are fundamental to completely evaluate its clinical prospective.

19.5.5 Berberine

Berberine (Fig. 19.3c) is an isoquinoline alkaloid found in various medicinal plants including the Chinese medical herb, *Coptis chinensis* Franch. This compound exhibits several pharmacological benefits, namely, antioxidant, anti-inflammatory, neuroprotective, antitumor, and antimalarial. Berberine has been reported to cross the blood-brain barrier and act on the central nervous system on several conditions, such as cerebral ischemic injury, AD, PD, depression, anxiety, HD, epilepsy, and convulsions (Lin and Zhang 2018). The regulation of protein kinase B (Akt/PKB)-related signaling, B-cell lymphoma 2 (Bcl-2), NF-κB, glycogen synthase kinase 3β (GSK3β), and also MAPK- and AMP-activated protein kinase (AMPK) pathways explain the antiapoptotic, anti-inflammation, and antioxidant properties of this compound (Lin and Zhang 2018).

Yuan et al. (2019) recently reviewed the data on neuroprotective activity by berberine against AD using animal models. It induced neuroprotective properties and memory-improving effects through different mechanisms by ameliorating intracellular oxidative stresses, mitigating neuroinflammations, triggering autophagy, guarding neurons against apoptotic cell death, and inhibiting enzyme (butyrylcholinesterase, acetylcholinesterase, monoamine oxidase A, and monoamine oxidase B) activity, hyper-phosphorylation of tau protein, and β-amyloid (Aβ) peptide production (Fan et al. 2019). The results available from studies in animal models encourage the development of clinical studies to effectively evaluate the effects of

berberine on AD human patients. However, some studies indicate that berberine has dual effect, neuroprotective and neurotoxic, in the treatment of nervous diseases depending on the dose, and therefore, the use of these compounds should be analyzed with caution.

19.5.6 Cannabidiol

Cannabidiol (Fig. 19.3d) is a non-psychotropic cannabinoid isolated mainly from *C. sativa*. The cannabis-based preparation Sativex®, which comprises equimolar contents of cannabidiol and Δ^9–THC, is currently used in the control of neuropathic symptoms of multiple sclerosis (Fernandez 2016). Cannabidiol recently attracts the interest of the scientific community due to its safe profile and vast range of bioactive effects including neuroprotective. It showed positive results in the management of several neurological conditions like HD, PD, AD, amyotrophic lateral sclerosis, and epilepsy (Watt and Karl 2017; Silvestro et al. 2019).

The neuroprotective action of this compound is mainly related to its anti-inflammatory and antioxidant properties. Most cannabinoids interact with cannabinoid receptors, but cannabidiol shows a low affinity for these receptors. The mechanisms by which these compounds exert its beneficial effects on neurological diseases are not totally known; however, the immunosuppressive and anti-inflammatory effects seem to be in part facilitated via the adenosine, serotonin, opioid, and non-endocannabinoid G protein-coupled receptors and other targets as ion channels and enzymes (Bih et al. 2015; Morales et al. 2017a). The anti-inflammatory potential of this compound has been demonstrated in mouse models and is modulated by different mechanisms, such as some pro-inflammatory cytokines and regulating cell cycle and immune cell functions. The strong antioxidant activity of cannabidiol is by modulating the expression of inducible nitric oxide synthase and nitrotyrosine as well as plummeting the production of ROS (Esposito et al. 2006). More controlled studies are essential to really assess the efficiency of this compound in neurodegenerative diseases (Morales et al. 2017b).

19.6 Concluding Remarks and Future Perspectives

The growing prevalence of neurodegenerative diseases worldwide, and the absence of effective treatments have led to extensive investigation in the last years concerning the search for new effective therapies. Plant compounds, including dietary phytochemicals have been widely studied in the last years concerning their beneficial effects for the prevention and treatment of these disorders. Furthermore, it is widely accepted that nutrition plays an important role in the occurrence and progression of many diseases. In the case of neurodegenerative diseases, lifestyle and diet intervention has been proposed as having positive effects to prevent and/or delay their progression. Among the diverse range of plant compounds, polyphenols, isothiocyanates, alkaloids, cannabinoids, and ginsenosides are some of the classes with

compounds showing relevant neuroprotective properties. Polyphenols, mainly present in fruits, vegetables, olive oil, wine, and tea, are the most important class of dietary phytochemicals. Several families of polyphenols from plants showed neuroprotective effects, due to a broad range of biological features. Resveratrol, curcumin, berberine, and cannabidiol are some of the most promising compounds with neuroprotective effects, and the extracts of *P. ginseng* and *G. biloba* are the most investigated. The protective role of phytochemicals includes several mechanisms of action including capacity of counteraction of oxidative stress, protection against neuronal damage caused by inflammation, and acetylcholinesterase, Aβ, and tau aggregation inhibitory capacities. Although there are many in vivo studies showing promising results, successful clinical trials in humans are scarce and in some cases with disappointing results. Also, the bioavailability and permeability across the blood-brain barrier of some plant compounds particularly of polyphenols is questionable. Anyway, the results obtained are sufficiently promising to justify future investigations aiming to translate the neuroprotective properties of plant compounds to the treatment of neurodegenerative diseases. Several strategies to overcome the limitations associated with plant compounds include the use of delivery systems and alternative administration routes and the engineering of structural analogues. Phytochemicals can be used in association with conventional therapies for the management of central nervous system disorders.

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Oroxylum indicum Vent. and Its Bioactive Compound, Baicalein Against Cancer Cells: Mechanisms of Action

20

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Contents

Abstract

Oroxylum indicum Vent. is an endangered plant, broadly known for its nutritional and medicinal properties. This plant is widely distributed throughout India and Southeast Asia. It is used in the Ayurvedic formulations to treat various health-related problems. Studies have revealed that *O. indicum* possesses various therapeutic properties, such as antioxidant, antimicrobial, anticancer, antidiabetic,

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hepato-protective, anti-inflammatory, nephro-protective, and cardioprotective properties. *O. indicum* contains a diverse array of bioactive principles that are belonging to different classes, such as phenolics, terpenoids, flavonoids, carotenoids, and anthocyanins. The major bioactive principles found in this plant are baicalein, oroxylin A, chrysin, scutellarin, and ellagic acid. Among these compounds, baicalein is the most potent compound with high anticancer activity against various cell lines. Presently, cancer is one of the most primary causes of mortality and responsible for nearly 9.6 million deaths, worldwide. Therefore, it is very much important to control cancer globally. Various treatments like chemotherapy, surgery, radiotherapy, and various anticancer medicines are available for cancers; however, they are not effective and cause severe side effects. Thus, herbal medicines are the best way to treat various cancers. This chapter mainly emphasizes on the anticancer activity of *O. indicum* plant extract and its major compound, baicalein. Further, mechanisms of action of baicalein on different cancer cells are highlighted.

Keywords
Anticancer · Dasamula · Baicalein · Oroxylin · Chrysin · Medicines

20.1 Introduction

The traditional medicines have been used for treating various human diseases, since the ancient time. The fossil records estimate that plants were being used as drugs nearly 60,000 years back (Ahad et al. 2012; Yuan et al. 2016). Plants play a significant role in human life as they provide food and shelter and possess therapeutic potentials conferred by the presence of numerous biologically active compounds (Kumara et al. 2011; Swamy and Sinniah 2016). According to the World Health Organization (WHO), more than 80% of world's population depend on traditional medicine for various health problems (Kumara et al. 2011; Swamy et al. 2016; Bhishma and Pawan 2018). Medicinal plants have a great importance in various traditional medicinal systems like Siddha, Ayurveda, homeopathy, Unani, Islamic medicine, traditional Chinese medicine, traditional Tibetan medicine, and allopathic medicine (Ahad et al. 2012; Achyuta et al. 2014; Arumugam et al. 2016; Mohanty et al. 2017). It is an established fact that bioactive compounds occurring in medicinal plants are responsible for treating various chronic and infectious diseases (Kumara et al. 2011; Swamy et al. 2016). Among various medicinal plants, *O. indicum* has a unique place in lieu of its various nutritional and pharmaceutical properties. The plant belongs to the family *Bignoniaceae* and is known by different names, such as tree of Damocles, broken bones tree, oroxylum, midnight horror, Indian caper, Indian trumpet flower tree, sonapatha, or shivnak (Rishu and Nutan 2017; Jayaram and Prasad 2008; Deka et al. 2013).

Oroxylum indicum is widely distributed throughout India and Southeast Asia, except in the western dry area. It is widely used in the herbal preparations by the people of China and Japan. Likewise, in India, herbs are part of traditional knowledge of various indigenous tribal communities. In Manipur, India, tribal communities like Anal, Kuki, Mao, Maram, Tangkhul, and Zeliangrong have a vast knowledge about the medicinal use of *O. indicum*. It is one of the ingredients of "Dasamula," an Ayurvedic product (Deka et al. 2013; Raghu et al. 2013; Dinda et al. 2015; Mahadevan et al. 2016). All parts like stem, root, leaves, and seeds of this plant have medicinal properties. This plant extract is safe for human consumption, if taken in a normal dosage (Deka et al. 2013). It shows various medicinal properties, such as antioxidant, antimicrobial, anticancer, antidiabetic, hepato-protective, anti-inflammatory, nephro-protective, cardioprotective, anthelmintic, antiulcer, immunomodulatory, and gastroprotective (Deka et al. 2013; Raghu et al. 2013; Dinda et al. 2015). It can also be used in the treatment of leprosy (Rajasekharan et al. 2017). These properties are due to the presence of various bioactive molecules like phenolics, terpenoids, carotenoids, flavonoids, anthocyanins, lipids, carbohydrates, flavors, and fragrances. The major bioactive principles identified and isolated include baicalein, oroxylin A, chrysin, scutellarin, ellagic acid, glycosides, and benzoic acid to name a few (Bhattacharje 2005, Ahad et al. 2012; Deka et al. 2013; Lalou et al. 2013; Roy et al. 2007; Rishu and Nutan 2017). Among these compounds, baicalein is considered to be the most common and ample flavonoids occurring in *O. indicum* and is known for its anticancer activity (Ahad et al. 2012; Rishu and Nutan 2017).

Cancer is one of the most leading causes of mortality and responsible for 9.6 million deaths worldwide (Akhtar and Swamy 2018a, b). According to WHO, one in every five men and one in every six women develop cancer during their lifetime, and one in every eight men and one in every 11 women die from the disease. Lung cancer, breast cancer, and colorectum cancer are the top three cancer types, causing higher mortality, globally (https://www.who.int/cancer/PRGlobocanFinal.pdf). There are various treatment approaches available today to treat cancers. These approaches include chemotherapy, surgery, radiotherapy, and various anticancer targeted therapies. However, these treatments may pose severe side effects and not be cost-effective (Ravichandra et al. 2018). Therefore, herbal medicines are considered as the one of the best ways to treat various cancers as they are reliable, inexpensive, effective, and not toxic (Kai et al. 2018). The foremost prominence of conventional drug innovations is to quest novel phytocompounds and their derivatives as cytotoxic agents. However, the advanced investigations by pharmaceutical industry mainly focus on anticancer lead molecules, their structural complexity, mechanisms of action, and pharmacological action against cancer cells (Rishu and Nutan 2017). Several studies have proved that *O. Indicum* and its major phytocompound, baicalein, exhibit antiproliferative activity against different cancer cells. For instance, in vitro studies have shown that baicalein inhibits several cancer cells, such as human promyelocytic leukemia (HL-60), human lung squamous carcinoma (CH27), and squamous cell carcinoma (SCC-4) cells (Roy et al. 2007; Ahad et al. 2012; Ying et al. 2016). Some of the experimentally proven mechanisms of action by baicalein

include the regulation of apoptosis via extrinsic and intrinsic pathways, induction of apoptosis by generating reactive oxygen species (ROS), and inhibition of angiogenesis, inducing cell cycle arrest (Chao et al. 2007; Maureen et al. 2016; Ying et al. 2016). However, the clear-cut mechanism of its anticancer activity still remains elusive. Currently, research focus is also toward the exploration of baicalein for its anticancer mechanisms of action. This chapter is mainly emphasized on the pharmaceutical aspects of *O. indicum* and its compound, baicalein, against different cancer cell lines. Moreover, different molecular actions arbitrated by baicalein against cancer cells are highlighted.

20.2 Botanical Aspects

20.2.1 Taxonomic Classification

The taxonomic classification of *O. indicum* (L.) Vent. is as follows (https://indiabiodiversity.org/species/show/16688):

Kingdom: Plantae.
Division: Magnoliophyta.
Class: Magnoliopsida.
Order: Lamiales.
Family: Bignoniaceae.
Genus: *Oroxylum.*
Species: *indicum.*

20.2.2 Vernacular Names

Oroxylum indicum plant is known by various names like broken bones tree, Indian calosanthes, Indian trumpet flower tree, midnight horror, oroxylum, and tree of Damocles (English); Hanyu pinyin: mù húdié, butterfly tree (Chinese); Bhut-vriksha, Dirghavrinta, Kutannat, Manduk (the flower), Patrorna, Putivriksha, Shallaka, Shuran or Son, and Vatuk (Sanskrit); Bhatghila (Assamese); Tona (Bengali); Tatelo (Nepalese); Tattuna, Tigadu, and Patagani (Kannada); Palaqapayyani, Vashrppathiri, and Vellappathiri (Malayalam); Corikonnai, Palai-y-utaicci, and Puta-puspam (the flower) (Tamil); Manduka-parnamu, Pampena, and Suka-nasamu (Telegu); Tayitu and Tetu (Marathi); Aralu, Shyonaka, Vatuk, and Son (Hindi); Bonglai (Malaysia); Phapni and Phonphonia (Oriya); Tatmorang (Punjabi); and Totila and Thotilav (Sinhala; Sri Lanka) (Deka et al. 2013; Rishu and Nutan 2017).

20.2.3 Origin, Geographical Distribution, and Morphology

Oroxylum indicum is native to the Himalayan foothills, Indian subcontinent, and also extending to Indochina, southern China, Bhutan, and Malaysia. It is found in the forest, Manas National Park in Assam, India. It is also reported from Sri Lanka (Ahad et al. 2012; Deka et al. 2013). It is a small- or medium-sized, 8–15 m-tall deciduous plant with light-brown soft spongy bark and corky lenticels. The leaves are 3–7 cm long, broad with 2–3 pinnate, and rachis very stout and cylindrical. The leaflets are 2–4 pairs and ovate or elliptic, acuminate, and glabrous. The flowers are numerous, fetid, in large erect racemes, reddish purple outside, and dull or pale pinkish yellow within. The fruits are flat capsules, up to 0.3 to 0.6 m long tapering to both ends. The seeds are flat, numerous, and 6 cm long with wings (Deka et al. 2013). In India, during August to February, flowers can appear any time depending on the climate (http://bioinfo.bisr.res.in/project/domap/plant_details.php?plantid=0091&bname=Oroxylum%20indicum). This tree is a night-bloomer and pollination naturally occurs by bats (Lawania et al. 2010).

20.3 Phytochemical Constituents

Oroxylum indicum is always of an interest for the researcher as it contains a large number of phytochemicals with medicinal values. Notably, all the parts of the plant are equally important. Various chemical constituents present in this plant are phenolics, terpenoids, carotenoids, flavonoids, anthocyanins, lipids, and carbohydrates. The specific bioactive compounds include baicalein, oroxylin A, chrysin, scutellarin, and ellagic acid (Roy et al. 2007; Yuan et al. 2008; Lawania et al. 2010; Zaveria and Jain 2010; Ahad et al. 2012; Deka et al. 2013; Rishu and Nutan 2017). The stem bark is found to contain flavones, such as baicalein (5,6,7-trihydroxy-flavones), chrysin (Chen et al. 2003; Harminder and Chaudhary 2011; Ahad et al. 2012; Rojsanga et al. 2017), oroxylin A (5,7-dihydroxy-6-methoxyflavone), scutellarin, ellagic acid (Harminder and Chaudhary 2011; Ahad et al. 2012), baicalein-7-*O*-glucoside, baicalein-7-*O*-diglucoside (oroxylin B) (Chen et al. 2003), scutellarin-7-rutinoside, traces of alkaloid, tannic acid, sitosterol and galactose, and biochanin A (Harminder and Chaudhary 2011). The leaves of *Oroxylum indicum* contain flavones and their glycosides, namely, baicalein and its 6- and 7-glucuronides, chrysin, scutellarin and its 7-glucuronides (Harminder and Chaudhary 2011; Ahad et al. 2012), anthraquinone, and aloe emodin (Harminder and Chaudhary 2011). The leaves of this plant are reported to contain quercetin 3-o-α-L-arabinopyranoside, 1-(2-hydroxyethyl)cyclohexane-1,4-diol, and apigenin (Ahad et al. 2012). The root bark contains biochanin A, ellagic acid, baicalein, chrysin (Harminder and Chaudhary 2011; Ahad et al. 2012; Barchung et al. 2018), scutellarin-7-rutinoside, and oroxylin A (Harminder and Chaudhary 2011). Traces of alkaloids, sitosterol, and galactose are also found in root. The root bark also has been reported to contain 2,5-dihydroxy-6,7-dimethoxyflavone and 3,7,3′,5′-tetramethoxy-4′-hydroxyflavone (Ahad et al. 2012). The fruits of *O. indicum* contain oroxylin A, chrysin, ursolic acid

(Harminder and Chaudhary 2011; Ahad et al. 2012), baicalein, and triterpene carboxylic acid (Harminder and Chaudhary 2011). The seeds have been reported to contain oils, alkaloids, benzoic acid, fatty acids, saponins, and flavonoids, such as chrysin, oroxylin A, oroxylin B, baicalein, terpenes, and tetuin. Another new flavones have been reported in the seeds, glucuronide-oroxindin and chrysin-7-O-diglucoside (Harminder and Chaudhary 2011; Mohapatra et al. 2018). The seed oil contains different types of compounds like caprylic, lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acids (Harminder and Chaudhary 2011; Ahad et al. 2012; Barchung et al. 2018). The structures of some major bioactive compounds are depicted in Fig. 20.1.

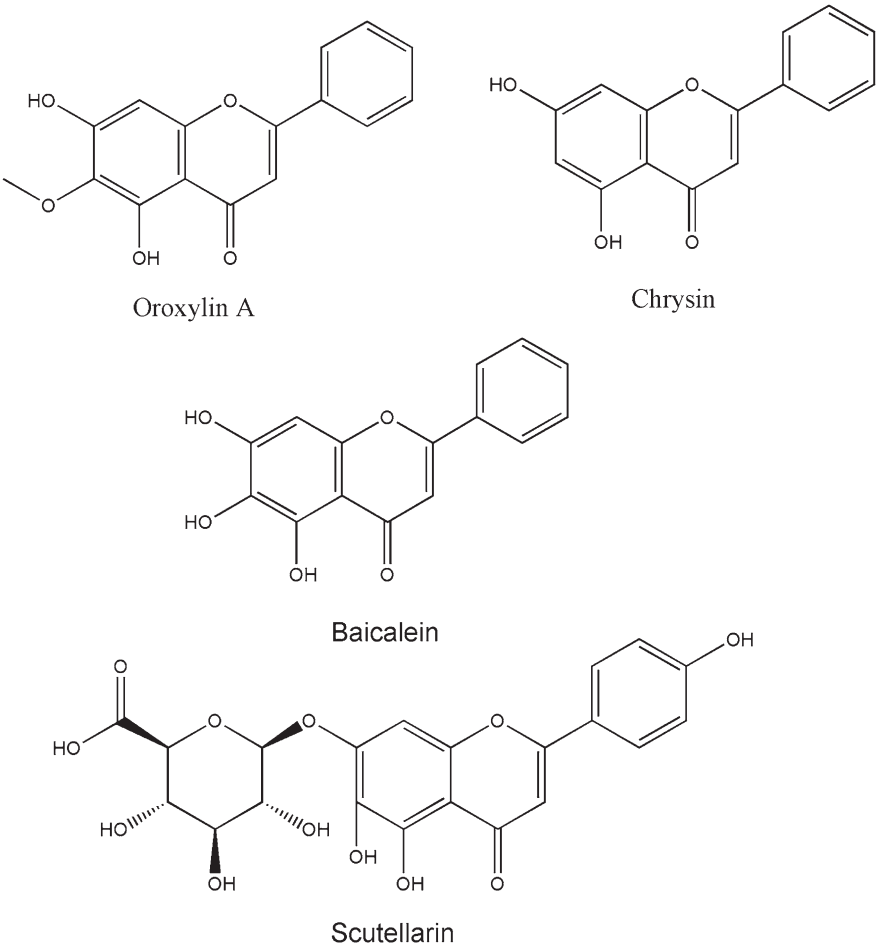

Fig. 20.1 Structures of some major bioactive compounds of *Oroxylum indicum*

20.4 Ethnobotany

Oroxylum indicum is habitually grown as an ornamental plant for its eccentric look. The farmers use this plant's branches or swordlike fruits to eliminate crabs in wet paddy fields. A paste prepared from *O. indicum* bark is smeared to injuries/wounds of animals for killing maggots. The seed paste is used to cure boils and wounds (Deka et al. 2013). The plant's flowers and fruits are consumed by people of Asian countries (Warrier et al. 1995; Harminder and Chaudhary 2011; Barchung et al. 2018). It has been utilized for centuries as a customary traditional medicine for preventing and treating several diseases including inflammation, jaundice, diarrhea, ulcer, fever, arthritis, cancer, and microbial infections (Biswas and Ghosh 1994; Khare 2004; Dinda et al. 2015). The stem and root bark of the plant are utilized for fever, intestinal worms, bronchitis, rheumatoid arthritis, leucoderma, inflammation, asthma, diarrhea, cough, etc. Bark decoction is taken for curing gastric ulcer, and a paste made of the bark powder is applied for mouth cancer, scabies, and other skin diseases. The seeds of the plant's fruit are used as expectorant and bitter tonic that can alleviate several human health problems. The dried powder of seeds is used by women to persuade conception. The essential oil of seeds is used by the fragrance industry. The root bark preparations are used in nasopharyngeal cancer, stomatitis, and tuberculosis (Harminder and Chaudhary 2011). The seeds are also used in the Chinese traditional system of medicines to treat cough, pertussis, pharyngitis, bronchitis, and other respirational disorders. In India, the preparations of Ayurvedic medicines employ this plant's fruits, seeds, bark, and leaves. The famous Ayurvedic formulation, "Dasamoola" contains this plant root extracts. This preparation is effective as an anti-inflammatory, anthelmintic, anti-leukodermic, anti-bronchitic, antianorexic, and antirheumatic. Also, it is used for the treatment of viral hepatitis, thyroid dysfunction, and gallstone disease. The plant roots are an ingredient of a well-known commercial product of Ayurveda, i.e., Chyawanprash (Dinda et al. 2015). Likewise, Chitraka Haritaki Avaleha, one more Ayurvedic formulation, also includes this plant parts as one of the ingredients, and it is used for treating cough, chronic rhinitis, asthma, hemorrhoids, helminthiasis, and digestive problems (Achyuta et al. 2014). The root bark of the plant is used for its stomachic property, and it is used as tonic which increases appetite (Rishu and Nutan 2017). The root bark decoction is used for diarrhea, dysentery, bronchitis, fever, intestinal worms, leucoderma, asthma, etc. (Ahad et al. 2012). The fruits are sweet acrid, stomachic, and anthelmintic. They are used to cure throat and heart disorders, jaundice, piles, and leucoderma (Deka et al. 2013; Rishu and Nutan 2017). It is a diaphoretic and hence used in rheumatism (Ahad et al. 2012; Rishu and Nutan 2017). The stem bark decoction is highly effective against nasopharyngeal cancer (Mao 2002). It is also used to cure diarrhea, dysentery, fever, hypertension, gastritis, epilepsy, etc. (Rishu and Nutan 2017). The seeds are found to be effective against throat infections, fever, pneumonia, respiratory problem, and hypertension (Rishu and Nutan 2017).

20.5 Anticancer Activities

Oroxylum indicum has drawn a considerable research interest, because of its antitumerogenic activity. The irregular proliferation of the cells is the characteristic feature of cancers. The cancerous cells have a huge capacity of invasion and replication (Ravichandra et al. 2018). The main focus of conventional drug discovery process is to search anticancerous compounds and cytotoxic agents (Rishu and Nutan 2017; Akhtar and Swamy 2018a, b). Nowadays, cancer is one of the leading causes of mortality worldwide. The main aim of cancer treatment is to remove cancer by various treatments like surgery, radiation, chemotherapy, and hormone therapy (Narayanaswamy and Swamy 2018; Lee et al. 2018). However, these treatments can have side effects. Traditional medicine has been used for various diseases from ancient time. Herbal treatment is one of the best methods to treat cancer due to the fact of easy availability, cost-effectiveness, and less or negligible side effects (Kai et al. 2018; Ravichandra et al. 2018). There are various mechanisms of action that are being reported by the phytocompounds exerting on different cancer cells (Narayanaswamy and Swamy 2018).

Apoptosis is one of the crucial events necessary to maintain tissue homeostasis (Thongrakard and Tencomnao 2010). There are a variety of mechanisms used by tumor cells to suppress apoptosis. So, induction of apoptosis in tumor cells is a specific therapeutic approach in chemotherapy. The literature survey suggested about various medicinal plant species with chemotherapeutic properties (Naveen et al. 2012). As discussed above, phytochemical studies have revealed that all the parts like root, bark, leaves, and fruits of *O. indicum* contain various phytochemicals and secondary metabolites. Among them, flavonoids, particularly baicalein, are the major constituents found in *O. indicum*. It is known to contribute in inhibiting several types of cancer cells (Ahad et al. 2012; Rishu and Nutan 2017). It was found that *O. indicum* extracts can control cancerous cells at various stages. However, the literature supported that baicalein also has multiple pharmacological properties like anti-inflammatory, antioxidant, and antiviral properties and is effective against heart diseases (Hui et al. 2016). Baicalein has in vitro inhibitory activity on different human cancer cells via different mechanisms. For example, it induces apoptosis and arrests the cell cycle to encourage the suppression of proliferating cancer cells (Jui et al. 2007; Narayanaswamy and Swamy 2018). Some of the reported molecular mechanisms of action of baicalein are discussed in the following section.

20.5.1 Effect of Baicalein on Apoptosis

20.5.1.1 Baicalein Regulates the Apoptosis Via Extrinsic and Intrinsic Pathways

Apoptosis is a programmed and ordered cellular process in our body. There are many morphological changes observed during apoptosis like DNA fragmentation, chromatin condensation, cell shrinkage, and blebbing (Ying et al. 2016). The suppression of apoptosis is the major cause of induction of cancers (Naveen et al.

2012). Cancer cells resist apoptosis and continue to proliferate uncontrollably, resulting in the increased tumor cell mass. Therefore, the main target to treat cancer is the signal pathways of apoptosis (Ying et al. 2016).

Apoptosis can initiate either through intrinsic pathway (mitochondria-mediated) or extrinsic pathways (receptor-mediated). The intrinsic pathway is raised by the release of cytochrome C from mitochondria to the cytoplasm, and it leads to the activation of caspase cascade through caspase-9 (Ying et al. 2016). The extrinsic apoptotic pathway is characterized by the binding of membrane death receptors with their ligands, such as the tumor necrosis factor-α (TNF-α), CD95L, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), leading to the initiation of caspase cascade through caspase-8. Baicalein can directly modulate the component of both intrinsic and extrinsic pathways of apoptosis (Ying et al. 2016). The B cell lymphoma-2 (BCL-2) family comprises a group of structurally related proteins that play an important role in intrinsic apoptotic pathway, and they are located predominantly on the mitochondria. BCL-2 family protein is divided into two major subgroups, the anti-apoptotic proteins and the pro-apoptotic proteins (Joseph and Anisha 2018). Pro-apoptotic proteins include Bax, Bad, and Bak, and anti-apoptotic proteins include Bcl-2, Bcl-xL, Mcl-1, and Bcl-w (Ying et al. 2016). Studies have suggested that BCL-2 family prevents or induces apoptosis (Aaron et al. 2015). The cell fate depends on ratio of pro-apoptotic to anti-apoptotic Bcl-2 family proteins (Ying et al. 2016; Joseph and Anisha 2018).

It was found that baicalein successfully induces apoptosis in SCC-4 (squamous cell carcinoma) human tongue cancer cells. The mechanism involves the increase of the levels of pro-apoptotic agents (Bax) and decrease of anti-apoptotic protein (Bcl-2) level, leading to increase in the ratio of pro- to anti-apoptotic family proteins. This induces the release of cytochrome C and other apoptogenic proteins from the mitochondrial membrane to cytosol, and it activates the caspase cascade and finally initiates apoptosis in the target cells. Baicalein also regulates the extrinsic apoptotic pathway by increasing the components like death receptor 5 (DR5) and other TRAIL receptors in cancer cells, suggesting the anticancer potential of baicalein (Ying et al. 2016).

20.5.1.2 Baicalein Regulates the Apoptosis Via Generating Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are small, highly reactive, and short-lived molecules. ROS can be the hydroxyl radicals (e.g., $OH^{\bullet}$), oxygen-derived free radicals (e.g., $O_2^{\bullet-}$), or non-radical molecules (e.g., H_2O_2) (Maureen et al. 2016). ROS plays an important role in apoptosis by acting as DNA-damaging agent (Barbora and Ale's 2011). The excess levels of ROS cause oxidative stress to the cell leading to damage of the proteins, nucleic acids, lipids, and also cell organelles like mitochondria and finally cause cell apoptosis (Maureen et al. 2016). Along with the generation of ROS, baicalein also causes endoplasmic reticulum (ER) stress, which leads to the activation of Ca^{2+}-dependent mitochondrial death pathways, and finally triggers apoptosis (Ying et al. 2016).

20.5.1.3 Baicalein Regulates the Apoptosis by MAPK Pathway

The mitogen-activated protein kinases (MAPKs) are the family of serine/threonine kinases that transduce many signals in response to a wide range of stimuli. The MAPK signaling pathways modulate gene expression, mitosis, proliferation, motility, metabolism, and programmed death, apoptosis. The subfamilies of MAPKs consist of the extracellular signal-regulated kinase (ERK), the c-Jun NH2-terminal kinase (JNK), and the p38 MAPK. It was found that among these subfamilies, ERKs are important for the cell survival, and JNKs and p38 MAPKs are involved in apoptosis (Teiji and Josef 2004). It was reported that baicalein plays an important role in regulating apoptosis by MAPK pathway. It increases the phosphorylation of p38 MAPK in bladder, lung, and breast cancer cells and diminished protein levels of survive in a member of the inhibitor of apoptosis (IAP) gene family (Ying et al. 2016).

20.5.2 Effect of Baicalein on Cell Cycle

Mutation in somatic cells is one of the main causes of cancer. However, a single mutation cannot transform normal cells to cancerous cells. Cancer results from the accumulation of mutations over a period of time. The main characteristic feature of cancer includes uncontrolled mitosis, which is involved in the disruption of cell cycle (Sandal 2002). Cell cycle is a series of events and composed of four stages like gap phase (G1), synthesis phase (S), gap 2 phase (G2), and mitosis phase (M). Cell cycle contains different checkpoints to monitor the events of all four stages (Roberta et al. 2016; Ying et al. 2016). The regulation of cell cycle checkpoints is controlled by cyclin-dependent serine/threonine kinases (CDKs), cyclins (CCNs), and inhibitors of cyclin-dependent kinases (CDKIs) that are required to control and regulate cell growth (Patrizia et al. 2014). The cell division is also regulated by the retinoblastoma protein phosphorylation (Henley and Dick 2012; Ying et al. 2016). CDKs consist of an active kinase subunit and regulatory subunits, called as cyclins. There are three classes of cyclins, namely, G1-S cyclins, S cyclins, and M cyclins, and they are primarily regulated by gene expression and protein degradation. When cyclins respond to mitogenic signals and unscheduled expressions, uncontrolled mitosis occurs, leading to cancers in humans (Sandal 2002).

The CDK inhibitors (CKI) arrest cell cycle in response to several antiproliferative signals. It was found that CKI plays an important role in preventing uncontrolled mitosis and tumor formation (Sandal 2002; Otto and Sicinski 2017). The most important transition in cell cycle is G1-S transition as it is highly regulated by many factors. When a cell enters from the late G1 phase to S phase, it is called as a critical point, where mainly CDKs (CDK4 and CDK6) and cyclins (D1, D2, and D3) are involved. G1 to S transition occurs through the regulation of phosphorylation and inactivation of the retinoblastoma tumor suppressor (Rb) pathways. The disruption of G1 to S transition causes cancers (Sandal 2002; Patrizia et al. 2014). Baicalein plays an important role to control cancers. Baicalein induces G1/S arrestin human lung squamous carcinoma (CH27) cells and osteosarcoma cells via

reducing the levels of CDK4 and cyclin D1 and increasing the level of cyclin E expression. It was found that baicalein controls prostate cancer by inducing G0/G1 arrest due to decreased levels of cyclin D1, cyclin D3, and phosphorylated retinoblastoma (pRB) protein (Ying et al. 2016).

20.5.3 Effect of Baicalein on Angiogenesis

Angiogenesis is the creation of new blood vessels from the existing ones (Vera et al. 2017). The formation of new blood vessels from preexisting blood cells occurs via the splitting and sprouting of endothelial cells. This process is a complex process and controlled by some biomolecules produced in the body. It is very important during embryo development, wound healing, and collateral formation (Mehdi and Shaker 2017; Malapelle and Rossi 2019). During cancer development, angiogenesis permits the creation of new blood vessels from existing ones. Studies said that cancer cells release molecules that induce the surrounding normal host tissue to form new blood vessels and promote the angiogenesis process (Nishida et al. 2006; Benazzi et al. 2014; Vera et al. 2017). Studies revealed that baicalein acts as inhibitors of angiogenesis. It was found that when cancer cells are treated with baicalein in a proper condition, the average number and length of sprouts formed by the endothelial cell aggregates were significantly decreased (Ying et al. 2016). In cancer cells, endothelial cells are very active and release many proteins like endothelial growth factor (EGF), vascular endothelial growth factor (VEGF), estrogen, fibroblast growth factor (FGF), interleukin-8 (IL-8), and tumor necrosis factor (TNF), which activates endothelial cell growth and motility (Mehdi and Shaker 2017). It was found that in ovarian cancer cells, baicalein effectively lowered the protein level of VEGF, hypoxia-inducible factor 1-alpha (HIF-α), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), and c-Myc. Apart from this, baicalein influenced phosphorylation of VEGF receptor 2 and ERK in human umbilical vein endothelial cells (HUVECs), which inhibit the formation of new blood vessel (Ying et al. 2016).

20.6 Conclusions and Future Prospects

Traditionally, *O. indicum* is a recognized medicinal herb used in folk medicines, such as Indian Ayurveda, Siddha, homeopathy, Unani, and traditional Chinese medicine. The documents have claimed that this plant can be effective in curing cancers, ulcer, dysentery, diarrhea, cough, inflammation, jaundice, and skin diseases and in wound healing. These claims were later proven by many research studies involving in vitro and in vivo models. However, many of the unknown biological properties possessed by *O. Indicum* extract or its sequestered bioactive compounds are yet to be proven scientifically. Till date, phytocompounds, such as baicalein, biochanin A, baicalin, chrysin, oroxylin A, iridoid, and 2,5-dihydroxy-6,7-dimethoxyflavone, are being identified in *O. indicum*, and several of these compounds are known to have

numerous therapeutic potentials. The major compound, baicalein, is being proved with superior anticancer effect against several cancer types. As discussed above, the mechanisms of action of anticancer effect mediated by baicalein include the regulation of apoptosis via extrinsic and intrinsic pathways, generating ROS, modifying MAPK pathway, arresting cell cycle, and effecting angiogenesis process. However, other possible molecular mechanisms are yet to be identified. In this direction, more focus should be given toward the research on baicalein compound for its anticancer effect and molecular mechanisms. In addition to studies related to toxicity aspects, more emphasis should be given to clinical trials to investigate the anticancer properties of baicalein in coming years.

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Indian Tropical Fruits and Their Bioactive Compounds Against Human Diseases

21

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Abstract

India has been blessed with a wide variety of wild and cultivated edible tropical fruits with unique taste and aroma/flavours. Fruits and vegetables are a rich reserve of nutritive fibres, vitamins, macronutrients and minerals, in addition to several phytochemicals. The Indian tropical fruits belong to diverse botanical groups, and some of the important edible fruits include mango, banana, papaya, citrus, guava, pineapple, litchi, sapota and pomegranate. Several minor and underutilized wild edible tropical fruits are also found throughout the country. Phenolic bioactive compounds, such as catechin, ellagic acid, epicatechin, epigallocatechin, anthocyanins, gallotannins, ellagitannins, gallic acid, sinapic acid, quercetin, resveratrol and kaempferol have been isolated from Indian tropical fruits, and these compounds are proven with medicinal and health-promoting properties. As a result, consumption of these fruits can be strongly allied to curtailed risk of various human diseases, including coronary heart diseases, diabetes and cancers. Antiproliferative activities, protection of cellular damage by free radicals, apoptosis and anti-inflammatory action are the main mechanisms by which fruits and vegetables are known to exhibit their chemoprevention and promote health.

Keywords

Tropical fruits · Bioactivities · Micronutrients · Phenolics · Antioxidants · Phytochemicals

21.1 Introduction

Tropical climatic zone (Fig. 21.1) is the geographical area extended between the Tropic of Cancer (23.5°N) and Tropic of Capricorn (23.5°S), occupying about 40% of the earth's surface (Val et al. 2005). Various types of fruits found within this region are called tropical fruits and composed of botanically diverse groups of plants. Countries within this region, including India are blessed with a wide variety of wild as well as cultivated edible fruits and vegetables with their unique taste and aroma/flavours. Fruits and vegetables are a rich resource of dietary fibres, vitamins, macronutrients, minerals and many phytochemicals, making them with the potential of providing a well-balanced diet (Lim et al. 2007; Mertz et al. 2009; Rufino

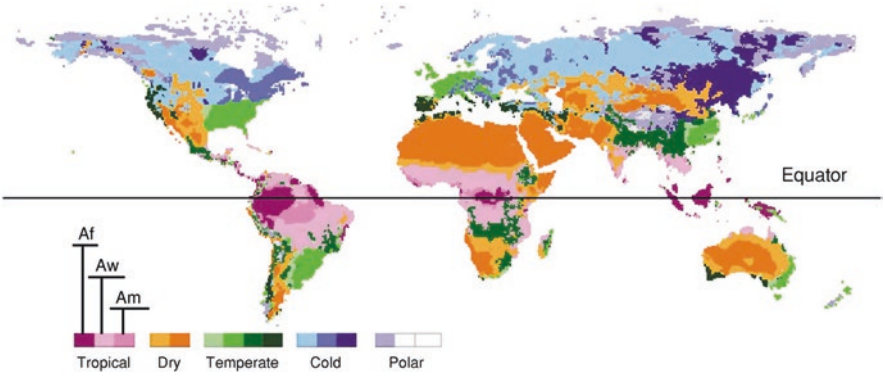

Fig. 21.1 Main types of tropical climates (*Reproduced with permission from Val AL, Almeida-Val VMFD, Randall DJ,* Copyright©2005 Elsevier Ltd)

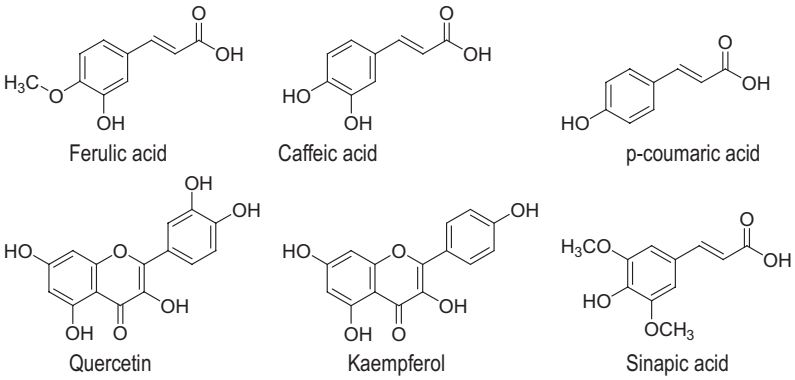

Fig. 21.2 Bioactive phytochemicals found in tropical fruits

et al. 2010). Phenolics and carotenoids (Figs. 21.2 and 21.3) are the major phytochemical constituents present in fruits. The health benefits of vegetables and fruits are because of the occurrence of these bioactive compounds (Singh et al. 2016a). Tropical fruits are rich in various phenolic acids, flavonoids and carotenoids. Different parts of the fruits as well as their by-products are also shown to contain abundant amount of these phytochemicals (Ajila et al. 2010; Singh et al. 2016b; Stafussa et al. 2018). However, the amount and types of phytochemical contents of tropical fruits differ widely between the species and cultivars and within the tissues (Rinaldo et al. 2010).

It is well-recognized now that lipid peroxidation and protein and nucleic acid impairment in cells are induced by free radicals, resulting in several degenerative conditions, such as inflammation, heart diseases and ageing (Alothman et al. 2009). The abundant antioxidants, including polyphenols, vitamin C and carotenoids present in vegetables and fruits are reported to help in inhibiting the incidence of

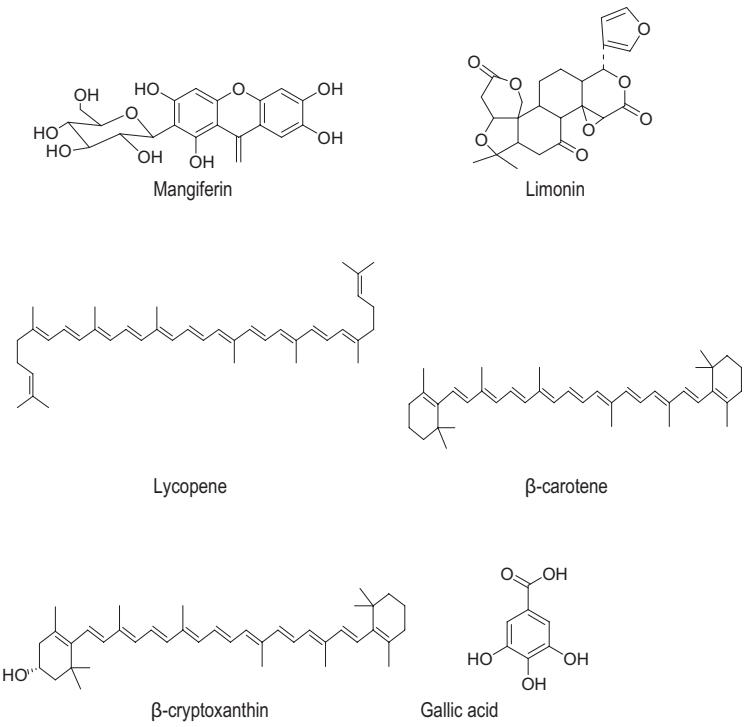

Fig. 21.3 Carotenoids and other phytochemicals present in tropical fruits

disorders (Lim et al. 2007). Several mechanisms have been proposed by which these dietary phytochemicals foster the antioxidant and other bioactivities. These mechanisms include antioxidant activity, reduction of oxidative stresses, regulation of DNA damage repair, suppression of cell multiplication and oncogene expression, stimulation of apoptosis, anti-inflammation and antiangiogenesis (Liu 2013). As a result, the health and nutritional benefits of more tropical fruits have been recognized over the years, leading to a gradual upsurge in the tropical fruit consumption, around the globe (Rufino et al. 2010). Considering the widespread availability of unexplored tropical fruits around the world, a vast expanse of scope remains open for the scientific investigations on the health and nutrition attributes of several underutilized tropical fruits.

India produces about 44 million tonnes of fruits every year, and is the second leading producer of world's tropical fruits, behind only to China in terms of annual fruit production. Majority of fruits produced in India include mango, banana, different citrus fruits, guava, grape, pineapple and apple (Khoje and Bodhe 2015). Thus, fruits produce is one of the major contributors to the Indian agriculture sector. Fruits and vegetables are central in the Indian diet system, since ancient times. In addition, owing to their nutritive and therapeutic belongings, several tropical fruits have also been employed in traditional medicinal practices, including Ayurveda and other

tribal medicinal systems (Mukundan and Narayanankutty 2017). Various studies on the bioactive components of these fruits and their potential mechanisms of action probed over the years have also established the potential health benefits of these Indian tropical fruits. Taxonomic classifications of major tropical fruits are given in Table 21.1. A detailed discussion on the bioactive components and bioactivities of commonly found Indian tropical fruits is presented in this chapter.

21.2 Bioactive Compounds and Bioactivities of Indian Tropical Fruits

21.2.1 Banana

Banana is a subtropical fruit, belonging to *Musa* genus (family, Musaceae). The basis of banana is believed to be the humid province of Southeast Asia, and its cultivation has been spread across the world (Arora et al. 2008). It is one of the common fruits, accounting for almost 16% of the global fruit production, and it is trailed by apple and orange (Table 21.2). India is known as the largest producer of banana fruit in the globe (30.47 M tonnes), trailed by China, Indonesia, Brazil, Ecuador and the Philippines (FAOSTAT 2017). All edible cultivars of banana belong to the genus *Musa* and are categorized into four parts according to the number of chromosomes present, which include *Australimusa*, *Callimusa*, *Eumusa* and *Rhodochlamys*. All over the world, over 300 different kinds of bananas have been grown and are classified depending upon the existence of a set of chromosome numbers and the amount of genomes of *Musa acuminata* (A) and *Musa balbisiana* (B) present. Examples of these hybrid genomes include plantains and dessert bananas, and their fruits differ by containing different amount of starch and sugar (Pereira and Maraschin 2015). *Musa acuminata* is the major source, where most of the edible bananas including *M. balbisiana* have been originated, and it belongs to *Eumusa* (Jones and Daniells 2018). The AA and AAA subspecies of banana have sweeter taste and are most commonly marketed, while the hybrid triploid subspecies (AAB, ABB, BBB) like plantains and cooking bananas contain large amount of starch. The most commonly cultivated species of *Musa* are *M. sapientum*, *M. cavendishii* and *M. paradisiacal.* Amongst them, *M. cavendishii* (AAA group) is considered the sweetest variety and contains less starch and is also called dessert banana (Zhang et al. 2005).

21.2.1.1 Bioactive Compounds

Various antioxidants, mainly carotenoids, vitamins and phenolics, such as catechin, epicatechin, gallic acid, lignin, anthocyanins and tannins are found to occur in the pulp of banana fruit. The content of total phenolic acids in *M. sapientum* banana was testified to be 7 mg/100 g fresh weight (FW) (Mattila et al. 2006). In *M. cavendishii* banana, gallocatechin was reported to be in large amounts in banana peel as compared to its pulp (Someya et al. 2002). Another study on two banana cultivars of China also reported the higher total flavonoid substances in banana peel compared to its pulp (Fu et al. 2018). The primary phenolic composites in these bananas

Table 21.1 Taxonomic classification of major tropical fruits

Common name	Division	Class	Order	Family	Genus	Species
Mango	Magnoliophyta	Magnoliopsida	Sapindales	Anacardiaceae	*Mangifera* L.	*Mangifera indica* L.
Pomegranate	Magnoliophyta	Magnoliopsida	Myrtales	Punicaceae	*Punica* L.	*Punica granatum* L.
Litchi	Magnoliophyta	Magnoliopsida	Sapindales	Sapindaceae	*Litchi* Sonn.	*Litchi chinensis* Sonn.
Pineapple	Magnoliophyta	Liliopsida	Bromeliales	Bromeliaceae	*Ananas* Mill.	*Ananas comosus* (L.) Merr.
Papaya	Magnoliophyta	Magnoliopsida	Violales	Caricaceae	*Carica* L.	*Carica papaya* L.
Banana	Magnoliophyta	Liliopsida	Zingiberales	Musaceae	*Musa* L.	*Musa acuminata* *Musa balbisiana*
Citrus	Magnoliophyta	Magnoliopsida	Sapindales	Rutaceae	*Citrus* L.	*Citrus limon,* *Citrus aurantifolia* *Citrus sinensis* *Citrus aurantium* *Citrus paradisi*
Guava	Magnoliophyta	Magnoliopsida	Myrtales	Myrtaceae	*Psidium* L.	*Psidium guajava* L.
Sapota	Magnoliophyta	Magnoliopsida	Ebenales	Sapotaceae	*Sapota* Mill.	*Manilkara zapota* L.

Table 21.2 Top ten producing countries of major tropical fruits in 2017 (FAOSTAT 2017)

	Production in tonnes					
				Mango,	Citrus	
Country	Banana	Papaya	Pineapple	Guava and mangosteens	Lemon and Limes	Oranges
India	30,477,000	5,940,000	1,861,000	19,506,000	2,364,000	7,647,000
China	11,170,000	1,057,101	1,576,405	4,791,271	2,316,876	8,564,425
Indonesia	7,162,685	875,112	1,795,986	2,566,046		2,295,325
Brazil	6,675,100		2,253,897	1,547,606	1,292,798	17,459,908
Ecuador	6,282,105					
Philippines	6,041,369		2,671,711			
Angola	4,301,880					
Guatemala	3,887,439					
Colombia	3,786,672	179,979	1,091,042			
Tanzania	3,484,788					
Mexico		961,768	945,210	1,958,491	2,528,174	4,629,758
Dominican Republic		869,306				
Nigeria		837,738	1,642,376			
Democratic Republic of Congo		214,836				
Cuba		189,086				
Venezuela		178,740				
Costa Rica			3,056,445			
Thailand			2,123,177	3,824,279		
Pakistan				1,685,304		1,585,090
Bangladesh				1,517,691		
Egypt				1,351,316		3,013,758
Malawi				1,323,680		
Argentina					1,676,000	
Turkey					1,007,133	1,950,000
Spain					923,192	3,357,163
USA					803,770	4,615,760
South Africa					446,468	
Iran					44,268	

include ferulic, salicylic, sinapic, p-hydroxybenzoic, gallic, p-coumaric, syringic, vanillic and gentisic acids. A conjugated form of ferulic acid was found to be the highest phenolic acid in *M. acuminata* (Russell et al. 2009).

Carotenoids are recognized to be the most important pigments found in plants, which are further categorized as carotenes (pure hydrocarbons) and xanthophylls (oxygenated derivatives of hydrocarbons). The fruit's peel is often the richest resource of carotenoids found in vegetables and fruits (van den Berg et al. 2000). Banana peels are also good sources of carotenoids. About 300–400 μg of carotenoids per 100 g of banana peel was reported from an investigation, involving the analyses of both alumina column chromatography and high-performance liquid

chromatography (HPLC), and the carotenoids identified from the banana peel include lutein, isolutein, α-carotene, β-carotene, auroxanthin, neoxanthin, violaxanthin, α-cryptoxanthin and β-cryptoxanthin (Subagio et al. 1996; van den Berg et al. 2000; Singh et al. 2016b).

Amination of ketones and aldehydes and decarboxylation of amino acids yield nitrogenous compounds, called as biogenic amines. Some of the biogenic amines like dopamine, serotonin and norepinephrine along with other phytonutrients are found in both banana pulp and peel, and are considered to be good for human health and responsible for imparting a state of well-being (Pereira and Maraschin 2015; Singh et al. 2016b). The serotonin and dopamine contents in banana seemed to vary amongst the different species; serotonin content ranges from 8 to 50 μg/g, while dopamine was found to be present at 42 μg/g in yellow banana pulp (*M. acuminata*), 54 μg/g in red banana pulp (*M. sapientum*) and 5.5 μg/g in plantain pulp (Feldman et al. 1987; Singh et al. 2016b). A study also reported the decreasing bioactive amine content in banana pulp with ripening and, rather than the pulps, the peels of banana were better source of biogenic amines such as serotonin, dopamine, histamine and tyramine (Borges et al. 2019). Other biogenic amines such as putrescine and spermidine were also found to be present in banana (Sanchez-Perez et al. 2018).

Banana pulps and peels are a rich resource of phenolic constituents, comprising different flavonoids like quercetin, myricetin, catechin, epicatechin and kaempferol. Additionally, the occurrence of vitamin C is also being reported from banana (Pereira and Maraschin 2015; Singh et al. 2016b; Septembre-Malaterre et al. 2016). Someya et al. (2002) showed that the content of gallocatechin in the peel and pulp of banana (*M. cavendishii*) was 158 mg/100 g dry weight (DW) and 29.6 mg/100 g DW, respectively. Several lipid compounds are present in banana pulp, predominantly in the form of fatty acids, sterols and steryl esters (Ghag and Ganapathi 2018). Plant sterols, which are considered functional foods responsible for lowering cholesterol (hypocholesterolaemic activity) in the blood, are occurring in banana at about 11.4 to 28% of the lipophilic extractives (Marangoni and Poli 2010; Vilela et al. 2014). Some of the phytosterols reported to be present in banana include sitosterols, stigmasterol, cycloeucalenol, cycloartenol, campesterol, 24-methylenecycloartenol, β-sitosterol and α-tocopherol (Knapp and Nicholas 1969; Qamar and Shaikh 2018; Vilela et al. 2014). The saturated fat contents in bananas are very low almost without any cholesterol (Ghag and Ganapathi 2018). Pazmino-Duran et al. (2001) identified different anthocyanidins like cyanidin, pelargonidin, malvidin, delphinidin, peonidin and petunidin in banana peels, and therefore considered banana waste as not only a better resource of natural pigments but also a helpful substance for the identification of anthocyanins.

Like other fruits, the pulp of bananas and plantains contains organic acids, including malic, glutamic and oxalic acids (Wyman and Palmer 1964). However, the acid and glucose contents of banana tend to change with ripening process. During its shelf life, the pH of banana fruit tends to increase due to a decrease in the total acid contents, while a strong rise in the glucose level was observed as the starch gets hydrolyzed (Taiti et al. 2015). The vitamin and mineral contents of

banana tend to change in view of the ripening or maturing process (Wills et al. 1984). It was reported that vitamin C and potassium were the nutritionally significant vitamin and mineral present in the banana pulp.

21.2.1.2 Bioactivities

Both the pulp and peels of different *Musa* banana fruits are considered to be medicinal by several traditional systems of practices around the globe (Tsamo et al. 2015). In Brazil, banana peels have been traditionally used to stimulate wound healing, especially burn wounds, due to its antiseptic properties as a result of its phytochemical constituents (Pereira and Maraschin 2015). The pulp contains potassium and iron at nutritious level and hence makes it as a beneficial source for the maintenance of normal blood pressure and in the treatment of anaemia (Singh et al. 2016b). As indicated in the bioactive components of bananas, both the peels and pulps are a rich source of antioxidants, including ascorbic acid (vitamin C), tocopherols (vitamin E), dopamine, carotenes, rutin and catecholamines that are essential for the human body. The superior free radical scavenging activity of banana peel extract over the extract of the pulp was demonstrated by establishing their scavenging capacity of free radicals like 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Kanazawa and Sakakibara 2000). The free radical scavenging and antitumor properties of banana peel extract were also demonstrated by researchers in the latest study (Kumar et al. 2019). Bananas are also rich sources of dietary neurotransmitters, such as dopamine and serotonin. Dopamine plays an essential part in different activities of the body, for example, coordinating body movement and motivation, while serotonin is important in modulating human behaviour and regulation of the gastrointestinal tract motility (Briguglio et al. 2018).

Provitamin A carotenoids are plentiful in different cultivars of banana, and as such, are potential food to ease the burden of deficiency of vitamin A (Englberger et al. 2003). Banana is recognized for having the antiulcer property. The antiulcerogenic agent in the pulp of green plantain banana (*M. sapientum L.* var. *paradisiacal*) was recognized as leucocyanidin, and this agent was observed to possess shielding effects against the erosion prompted by aspirin (Lewis et al. 1999). Studies have shown that this antiulcer bioactive compound is hydrophilic, which converts into inactive compound in ripe banana (Lewis and Shaw 2001). Banana is also thought to have efficacy towards diseases like atherosclerosis and hyperlipidaemia. The effect of banana consumption was studied on hypercholesterolaemic and diabetic subjects for 12 weeks, and the daily consumption of banana fruit as breakfast was found to significantly reduce the fasting blood glucose level plus LDL-cholesterol and HDL-cholesterol ratio in hypercholesterolaemic subjects (Cressey et al. 2014). Interestingly, the flavonoids from unripe banana fruit extract exert hypolipidaemic action by reducing the level of different lipids like phospholipids, cholesterol, triglycerides and free fatty acids in the brain, kidney, liver and serum of an experimental animal (Krishnan and Vijayalakshmi 2005). Banana contains small quantities of fats and high contents of carbohydrates, and hence it is normally used in diets with low fats. Gasster (1963) recommends banana for weight loss and treating obesity, and it has also been employed for managing diarrhoea in infants.

21.2.2 Citrus Fruits

The genus *Citrus* L. belongs to the family, Rutaceae. It is another impressive fruit crops of the world (Mehl et al. 2014). Different *Citrus* species have been grown in the Southeast Asia over thousands of years, and based on the enormous diversity of *Citrus* plants over a small area of land, Northeast India has been assumed to be the native home of *Citrus* fruits (Ollitrault and Navarro 2012; Sharma et al. 2004). Some of the members of the genus *Citrus* include lime (*C. aurantifolia)*, lemon (*C. limon)*, grapefruit (*C. paradisi)*, sweet orange (*C. sinensis)*, bitter orange (*C. aurantium)*, mandarins (*C. reticulata)*, pomelos (*C. grandis)* and citrons (*C. medica*) (Kefford 1960).

China, Mexico, Brazil and the United States are amongst the earth's topmost citrus-growing nations. Citrus trees are evergreen, and the size varies depending on the species. The fruits are mostly round in shape; however, the size, flesh and skin colour vary according to the *Citrus* varieties. For instance, lime and pomelo have greenish skin; however, lemon has a dark yellowish flesh and skin. Fruits of *Citrus* species can be consumed as a fresh fruit or juice and also used in many dishes and beverages. *Citrus* fruits are a rich resource of phytochemicals and possess numerous medicinal values, such as flavonoids, vitamins, minerals, essential oils, dietary fibre, limonoids and carotenoids, which make major contributions in food supplements and functional fruits (Gonzalez-Molina et al. 2010). Amongst the citrus cultivars, oranges contribute to more than 50% of the world's citrus fruit production, which is trailed by fruits of easy peeling, such as mandarins, clementines and tangerines, lemons, limes, grapes and pomelos (Barreca et al. 2014).

21.2.2.1 Bioactive Compounds

More than 170 antioxidant compounds have been reported from citrus fruits that include vitamins, i.e., vitamins A, B1, B2, B3, C and E; phenolic compounds; minerals; pectin; and terpenoids (Zou et al. 2016). The peels and pulps of citrus fruit are rich sources of folic acid, ascorbic acid, pectin and potassium (Rafiq et al. 2018). The existence of these flavonoids and other polyphenolics along with carotenoids, mineral compounds and essential oils in citrus fruits provides numerous beneficial pharmacological activities that are helpful in the prevention of several degenerative diseases (Wang et al. 2014).

Several flavonoid compounds, including polymethoxylated flavones (PMFs), flavanones and flavonols have been identified from seeds and peels of citrus fruits. All citrus fruits, including oranges, lemon and grapes are reportedly containing flavanones, which contribute to the flavour of citrus fruits and peels (Panche et al. 2016). Citrus flavanones occur in both aglycone and glycoside forms. Hesperidin and naringenin are the most important aglycone form of flavonoids, while the two types of glycoside forms include rutinosides and neohesperidosides (Peterson and Dwyer 1998). The compositions of bioactive compounds in the seeds and peel of citrus fruits may not be same all the time. For instance, the seeds of lemon predominantly contain hesperidin and eriocitrin, whereas the peel contains more of neoeriocitrin, naringin and neohesperidin, which can be extracted from the peel in sufficient

quantity (Bocco et al. 1998; Nogata et al. 2006; Rafiq et al. 2018). Sour orange is a good source of neohesperidin and naringin which are useful in the preparation of sweetening agents (Bocco et al. 1998). Limonoids are chemically related to triterpene derivatives, and are one of the reasons for the bitter taste of citrus fruits. About 36 limonoid aglycones have been reported from citrus fruits (Hasegawa et al. 1996). Nomilin and limonin are the most common limonoids found in citrus fruits (Miller et al. 2007). Limonin is accountable for the late bitterness in citrus fruits, and it was firstly isolated from citrus seeds by Bernay in 1841 (Higby 1938), but all the other limonoids were isolated over the last 6–7 decades. Limonoids occur only in Rutaceae plants (lemon, orange, grapefruits) and mahogany family, Meliaceae (neem); the solid flesh and peels of citrus fruits may contain about 500 mg of limonoids per 100 g of the fruit (Turner and Burri 2013).

At least 19 elements are detected in citrus plants, out of which copper, iron, manganese, zinc and selenium were described to possess antioxidaive activities (Zou et al. 2016). Anthocyanins, the compound responsible for variety of pigments in fruits and vegetables, are also found to be present in different citrus fruits (Ballistreri et al. 2019). Citrus peels are also considered to be a good source of pectin and dietetic fibres (Baker 1997; Terpstra et al. 2002).

21.2.2.2 Bioactivities

The presence of phytocompounds like polyphenols, minerals, carotenoids, essential oils and other nutritive fibres in citrus fruits contributes to their beneficial properties in health and nutrition. These components, especially phenolic flavonoids, such as polymethoxyflavones in the peels, have been identified to have a vital role in arresting neurodegenerative diseases and cancers (Wang et al. 2014; Baliga et al. 2018). The fresh citrus fruits are popular for their rich vitamin C content, which is a strong antioxidant that protects cell damages from free radicals. It prevents degenerative diseases including cancers and cataracts. In addition, it promotes the formation of collagen, exhibits wound-healing property, and strengthens blood vessels, bones and ligaments (Carr and Frei 1999). Vitamin E, occurring chiefly in citrus fruit peels and seeds along with selenium, defends the damage of mitochondria from free radical species and lipid peroxidation of the cell membranes (Zou et al. 2016). Various flavonoid compounds in citrus fruits possess several advantageous properties, such as anticancer, antiviral, antioxidant and anti-inflammatory actions; prevention of platelet aggregation; and decrease fragility of capillaries (Benavente-García et al. 1997; Baliga et al. 2018). Flavonoids have the capability to quench singlet oxygen and act as chelating agent against metals like iron and copper (Hagerman et al. 1998).

The bioactive hesperidin and naringin isolated from citrus fruits have been identified to exhibit hypoglycaemic as well as hypolipidaemic activities in type 2 diabetic animal models. The mechanism by which these compounds induced the antidiabetic and antihyperlipidaemia was found to be the alteration of glucose-regulating enzymes and regulation of fatty acid and cholesterol metabolism, respectively (Jung et al. 2006). Similar findings were also reported recently for hesperidin in streptozocin-stimulated animal model of diabetic myocardial infarction (Rekha et al. 2019). Flavonoids can shield DNA from damage by absorbing ultraviolet

radiation (Tripoli et al. 2007). Investigations have reported the capability of rutin and naringenin, the aglycone of naringin to prevent DNA damage against UV-B radiation (Kootstra 1994).

Glutathione S-transferase (GST) is an important enzyme that catalyzes the conjugation of reduced glutathione to xenobiotic substrates. The presence of furan moiety in a compound or natural products induces GST activity (Lam and Sparnins 1987). Citrus limonoids, such as limonin and nomilin contain this furan moiety in their structure making it potential GST-inducing anticarcinogen compounds. A study in mice indicated nomilin as a potent inducer of GST enzyme in the small intestinal mucosa indicating its capacity to inhibit chemically induced carcinogenesis (Lam et al. 1989). Citrus limonoid glucosides, such as limonin 17-β-D-glucopyranoside, obacunone 17-β-D-glucoside, nomilinic acid 17-β-D-glucopyranoside and deacetylnomilinic acid 17-β-D-glucopyranoside were examined for their antioxidant and cytotoxic capacities against neuroblastoma cells, and all of them exerted antioxidant and cytotoxic activities by inducing apoptosis (Poulose et al. 2005). Citrus limonoids, including limonin, limonin methoxime, limonin oxime, obacunone, obacunone glucoside, nomilinic acid glucoside, deacetyl nomilinic acid glucoside and defuran nomilin were found to exhibit antiproliferative activity against breast cancer cells MCF-7 through caspase-7-dependent pathways (Kim et al. 2013). At higher concentrations (100 μg/mL), limonoids were also found to induce apoptosis in MCF-7 cells (Tian et al. 2001). Limonexic acid is isolated from sour orange (*C. aurantium* L.), and found to be effective apoptosis-inducing agent in human colon cancer cells (HT-29), while being non-toxic to non-cancerous cells (Jayaprakasha et al. 2010).

Pectins are characteristic polysaccharide components of citrus peels. The health benefits of citrus pectins include wound healing, immune complement activation, homeostasis and avoidance of chronic diseases, including cardiovascular diseases and diabetes (Liu et al. 2002). Pectin obtained from tangerine, grapefruit, lemon and orange had been found to prevent the binding of fibroblast growth factor (FGF) to FGF receptor demonstrating its antigrowth factor activity (Liu et al. 2001). Citrus peel extracts have also been reported to possess inhibitory action on the secretion of pro-inflammatory mediators, such as nitric oxide and prostaglandin E2, thereby exhibiting their anti-inflammatory activity (Huang and Ho 2010).

21.2.3 Pineapple

Pineapple (*Ananas comosus* L.), in the family of Bromeliaceae is amongst the top three main tropical and subtropical fruits worldwide, and South America is believed to be its place of origin (Da Silva et al. 2013). *Ananas* was the original name of pineapple which comes from Tupi word *nanas* meaning 'pine' and *comosus* meaning 'tufted' signifying the texture of the fruit stem (Hassan et al. 2011). It is also likely that the European explorers derived the name pineapple due to the similarity of the fruit to pinecones. In terms of production, Costa Rica leads the global pineapple production followed closely by countries including the Philippines, Brazil, India and Indonesia (Karmakar and De 2019).

About 30 different cultivars of pineapple are available around the globe and have been classified into four classes, namely, the Red Spanish, Pernambuco, Queen and Smooth Cayenne (Wali 2019). The Smooth Cayenne cultivars are the most popular pineapple around the world, due to its excellent qualities, and almost all fresh fruits and canned products of pineapples are made from this cultivar (Reinhardt et al. 2002). Pineapple may be used for preparing jams and juices, and it may also be taken as slices, chunks and fruit salad.

21.2.3.1 Bioactive Compounds

The excellent taste and flavours of pineapple have been accredited to its higher level of sugar as well as the balance between sugar and the acid levels (Sun et al. 2016). One of the important determinants of pineapple quality is its sugar content. Glucose and fructose are the predominant carbohydrates in the early fruit development, and at about 6 weeks prior to harvesting, sucrose starts to accumulate at a high rate in the fruitlet (Chen and Paull 2000). The principal organic acid in pineapple was found to be citric acid, which accounts for about 62% of the whole organic acids present in the fruit. Malic acid, tartaric acid and acetic acid were also observed to occur in the fruit (Lu et al. 2014; Sun et al. 2016). Various factors, including the pineapple clone, fruit maturation and growing conditions are known to cause variation in acidity and sweetness of the pineapple (Saradhuldhat and Paull 2007). The overall protein content is low; however, it contains an important component bromelain which has an important application in food industry (Hassan et al. 2011). The major minerals in pineapple were reported to be calcium, potassium and magnesium (Lu et al. 2014).

The specific attractive flavour in pineapples is the result of its various volatile and non-volatile components. About 343 volatile constituents have been identified and are testified to influence the flavour of pineapple. The major volatiles include esters, alcohols and phenols, aldehydes, ketones, lactones and terpenoids (Montero-Calderon et al. 2010). Even for the same pineapple hybrid, the volatile components seem to vary amongst different varieties (Zheng et al. 2012). The pivotal flavour or odorants in fresh pineapple fruits contributing to its aroma were identified to be ethyl 2-methylpropanoate, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, ethyl 2-methylbutanoate, 1-(*E,Z*)-3,5-undecatriene and methyl 2-methylbutanoate (Tokitomo et al. 2005). A study has provided a strong confirmation that accumulated aromatic compounds in pineapple enhance its flavour intensity, sweetness and consumer's acceptance (Schulbach et al. 2007).

Pineapples are a rich resource of vitamins, i.e., vitamins A, B and C (Hossain and Mizanur Rahman 2011). The vitamin C content was reported to be 47.8 mg/100 g of fresh weight (FW); niacin and pantothenic acid were estimated at 0.500 mg and 0.213 mg per 100 g of the fresh fruits, respectively (Wali 2019). The vitamin contents and their concentrations vary with the part and varieties of the fruit. Amongst different varieties tested, the highest amount of vitamin C was contained in the fresh juice of *A. comosus*, while the presence or absence of vitamin B_6 and vitamin B_{12} depends on the variety of pineapple (Sun et al. 2016).

Different varieties of pineapples are rich sources of various polyphenols (De Oliveira et al. 2009; Hossain and Mizanur Rahman 2011). The major phenolics reported from the fruit include *p*-coumaric acid, ferulic acid, caffeic acid,

sinapic acid, syringic acid, salicylic acid, tannic acid, tyramine, myricetin, gallic acid, catechin and epicatechin (Wen and Wrolstad 2002; Steingass et al. 2015; Dominguez et al. 2018). β-Carotene is one of the vital antioxidant compounds found in pineapple, and its concentration tends to increase with ripening of the fruit (Dominguez et al. 2018).

21.2.3.2 Bioactivities

The fruit and stem of pineapple contain a proteolytic enzyme called bromelain, which has outstanding pharmacological or medicinal properties. This compound isolated from pineapple is considered to be responsible for various therapeutic activities of pineapple including anti-inflammatory; regulation of immune responses; inhibition of platelet aggregation; and fibrinolytic, wound-healing and anticancer activities (Heinicke et al. 1972; Pavan et al. 2012; Sun et al. 2016). Bromelain is also considered to be capable of improving digestion in human (Devakate et al. 2009). A comparison was made between nonsteroidal anti-inflammatory drug diclofenac and combination of bromelain, rutin and trypsin in 103 patients suffering from osteoarthritis. The results showed reduced inflammations and pain, and the enzyme-rutin combination was considered as effective as diclofenac in alleviation of the pain associated with osteoarthritis (Akhtar et al. 2004).

Bromelain isolated from the stem of *A. comosus* was demonstrated to exhibit antitumour activity against P-388 leukaemia, sarcoma, Ehrlich ascites tumour, Lewis lung carcinoma and ADC-755 adenocarcinoma cells (Baez et al. 2007). In fact, the antitumour effect of bromelain was found to be higher than 5-fluorouracilinanimal model. The in vitro evaluation of anticancer activity of bromelain, obtained from fresh pineapple juice, on cancer cell lines, ovarian (A2780) and colon (HT29), showed the efficacy of bromelain in suppressing the growth and formation of cancer cells without hampering the normal cells (Gani et al. 2015). The antimicrobial effect of bromelain from pineapple has also been demonstrated against various potent periodontal pathogens, such as *Streptococcus mutans, Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis* and *Porphyromonas gingivalis* (Praveen et al. 2014).

The antioxidant compounds in pineapple possess scavenging activity towards free radical species (De Oliveira et al. 2009). Various types of phenolic compounds, vitamin C and β-carotene present in pineapple are considered responsible for the free radical scavenging activity. Indeed, the magnitude of antioxidative property is interconnected with the total amount of phenolics present in the sample (Hossain and Mizanur Rahman 2011). Dietary fibres present in pineapple contribute to the regulation of normal bowel movements. The presence of various minerals in pineapple including manganese, calcium, potassium, magnesium and phosphorus is essential for maintaining human nutrition (Beattie and Quoc 2000).

21.2.4 Papaya

Papaya (*Carica papaya* Linn.) belongs to family Caricaceae, and is one of the most important tropical fruits with huge demand in the international market.

Papaya is believed to be a native of tropical America; however, it is now cultivated and produced by tropical countries worldwide including India (Pino 2014). Due to its highly nutritious constituents, especially vitamin A, papaya has become an important dietary source prescribed to overcome several preventable diseases, such as xerophthalmia and anaemia (Schweiggert et al. 2011). Like any other fruits, the bioactive components in papaya and their subsequent bioactivity also depend on various factors including the cultivar types, the growing conditions, postharvest treatment, ripening stage and climates (Gayosso-García Sancho et al. 2017; Sangsoy et al. 2017).

21.2.4.1 Bioactive Compounds

The latex of an unripe papaya fruit contains three cysteine protease enzymes, namely papain, chymopapain and papaya proteinase III (Zucker et al. 1985). Chymopapain is known to be the most abundant protein in the latex, which was used in the non-surgical treatment of prolapsed intervertebral discs (Buttle and Barret 1984; Zucker et al. 1985). A study had shown that obtaining a good yield of these protease enzymes from the papaya latex depends on the source of latex and the methods followed for the isolation and crystallization (Kimmel and Smith 1954).

Wide ranges of phenolics, vitamins, provitamins and carotenoids have been identified in papaya fruits from different regions. Some of the phenolic compounds and organic acids reported from papaya fruit juice include caffeic acid and its derivatives, L-ascorbic acid, malic acid, citric acid, protocatechuic acid-O-hexoside, apigenin-O-pentoside, manghaslin, caffeoylmalic acid, *p*-coumaric acid, quinic acid, ferulic acid, rutin, coumarylglucaric acid, feruloylmalic acid, feruloylglucaric acid, quercetin, methyl gallic acid, sinapic acid and isorhamnetin (Spinola et al. 2015). Rivera-Pastrana et al. (2010) reported caffeic acid, rutin and ferulic acid to be the major phenolic compounds present in the mesocarp tissue of papaya. The content of phenolics on the fruit flesh and skin was reported to vary with the stages of ripeness. The *p*-coumaric acid, ferulic acid, and caffeic acid contents of the fruit skin tend to decline with maturity, while the vitamin C and carotenoid (lycopene, β-carotene, β-criptoxanthin) contents in the flesh increase with ripening (Gayosso-García Sancho et al. 2011). Papaya represents a rich source for vitamins C and A and holds first rank in vitamin contents per 100 g flesh out of 13–17 other fresh fruits (Gebhardt and Thomas 2002; Udomkun et al. 2015). The total carotenoids in a fully ripened papaya may range between 5414 and 6214 μg/100 g of the fruit weight, and as a result of its abundant vitamin A precursors, the fruit flesh of papaya is considered to be as nutritious as containing 132–166 μg retinol equivalents per 100 g of the fruit (Schweiggert et al. 2011). The red-fleshed papaya fruit has a high level of β-carotene compared to yellow-fleshed fruit, but β-cryptoxanthin content is same in both red-fleshed and yellow-fleshed fruits (Chandrika et al. 2003). These carotenoids are lipophilic in nature and accountable for the flesh colour, which in turn plays an important part in consumer's acceptance of the fruit (Gayosso-García Sancho et al. 2011). Minerals like sodium, potassium, calcium, magnesium and phosphorus are also found to be present in papaya fruits (Wall 2006).

The non-volatile organic acids in the pulp of papaya include citric, malonic, malic, tartaric and succinic acids, whose pH is in the range of 4.5–5.9 (Hernandez et al. 2009; Spinola et al. 2015). About 80% of acidity value in papaya is contributed by ascorbic acid, and the rest of 20% is due to citric acid, malonic acid, succinic acid, ketoglutaric acid and fumaric acid (Bron and Jacomino 2006).

Papaya has its characteristic flavour and odour, for which its volatile components contribute significantly. Several studies had been conducted to identify these volatile components, and more than 100 volatile constituents in papaya have been recognized (Pino 2014). According to their volatile components, papaya can be grouped into ester-rich chemotypes like Red Maradol cultivars from Cuba and terpene-rich chemotypes, such as Hawaiian solo papayas containing high concentrations of terpenes including linalool and benzyl isothiocyanate (Lieb et al. 2018). A study to monitor the release of volatile compounds from papaya during the four ripening stages indicates the release of linalool, benzyl isothiocyanate and phenylacetonitrile throughout the four stages, and on approaching the full ripeness, the production of linalool was found to increase dramatically (Flath et al. 1990).

21.2.4.2 Bioactivities

Different parts of papaya plant including the fruits, leave, latex, flowers, seeds and barks are known for their medicinal values and have been used in the treatment various. The use of papaya fruit as topical ulcer dressings is very popular in Jamaica (Hewitt et al. 2000). Studies conducted in a mouse burn model revealed that the papaya latex exerts its wound-healing property through synthesis of collagen by increasing hydroxyproline level (Gurung and Skalko-Basnet 2009). Aqueous extracts of both seeds and leaves exert antifungal action against *Colletotrichum gloeosporioides* (Bautista-Banos et al. 2002), and the extract from the leaves of Marado cultivar also has antifungal activity against *Fusarium* spp. with a MIC_{50} of about 625 mg/mL (Chavez-Quintal et al. 2011). Both water and organic extracts of papaya seeds showed antihelminthic property against *Caenorhabditis elegans* (Kermanshai et al. 2001).

Lycopene, a bright red carotenoid, has been shown to have higher free radical scavenging activity when compared to β-carotene and is considered to reduce the chances of onset of cancers in the lung, prostate and stomach (Di Mascio et al. 1989). As the red-fleshed cultivars of papaya, such as Sunrise and SunUp, are reported to contain high amount of lycopene, they can be expected to contribute significantly in the promotion of health benefits than their vitamin activity (Wall 2006). Many researches have also suggested the anticancer activities of papaya fruits. Rahmat et al. (2002) studied the antitumour activity of pure lycopene and lycopene isolated from the juice of papaya and watermelon and concluded that both papaya juice and lycopene extracted from papaya resulted in cell death of hepatic cancer cell line, HepG2 (Nguyen et al. 2013). The examination of the antiproliferative activity of water extract of papaya leaves on solid tumour cells also reported the inhibition of the cell multiplication in the examined cells and suggested apoptosis as the main mode of action (Otsuki et al. 2010).

Papaya leaf extract has commonly been employed in Indonesia and Malaysia in the management of thrombocytopenia. The antithrombocytopenic activity of papaya leaf extract was studied on busulfan-stimulated thrombocytopenic Wistar rats (Zunjar et al. 2016). The alkaloids, especially carpaine in leaves, were found as the reason for antithrombocytopenic activity of the leaf extract. A study on Swiss albino mice also showed the increase thrombocyte counts in mice treated with the suspension of papaya leaves, and this may help explain the use of papaya leaves in the treatment of dengue infections (Sathasivam et al. 2009). Similarly, Yunita et al. (2012) administered the papaya leaf extract capsules to patients with dengue fever and found an elevation in the blood platelet counts at a significant level, stabilizing the hematocrit level along with the overall reduction in hospitalization period.

Fermented papaya preparation has been reported to elicit various bioactivities including favourable modulation of parameters affecting the haematological, immunological, inflammatory, vascular and oxidative stress damages (Aruoma et al. 2010). The papaya leaf extract was also reported to exhibit hypoglycaemic activity in animal models causing a decline in the level of glucose, triglycerides and transaminase in the serum (Juarez-Rojop et al. 2014). An antifertility compound, 1,2,3,4-tetrahydropyridin-3-yl-octanoate, has been sequestered from the papaya seeds (Julaeha et al. 2015).

21.2.5 Mango

Mango (*Mangifera indica* L.), belonging to Anacardiaceae family is one amongst the popular tropical fruits worldwide. It is known for its excellent flavour, attractive colour and highly nutritious contents (Fig. 21.4) including vitamins and carotenoids (Rumainam et al. 2018). India is believed to be the origin of mango, and more than 1000 cultivars of mangoes are available in India alone, reflecting its diversity (Radha et al. 2018; Saleem Dar et al. 2016). The cultivation of mango in India started about 4000 years ago, and the fruit still occupies an important place in the Indian fruit market (Ribeiro and Schieber 2010).

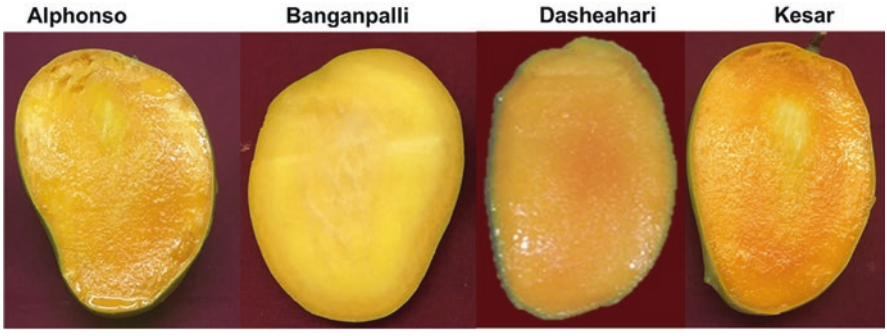

Fig. 21.4 Different mango varieties at ripe stage (*Reproduced with permission from* Singh et al. (2017), Copyright©2017 Springer Nature)

21.2.5.1 Bioactive Compounds

Apart from the fruit, the peels and seeds of mango are a source of natural antioxidant and other bioactive principles. The bioactive components seemed to differ in the plant parts and the stages of ripening (Ajila et al. 2007; Saleh and El-Ansari 1975). The phytochemical contents of the mango pulp include gallic acid, *p*-coumaric acid, ferulic acid, vanillic acids, m-coumaric acid, ellagic acid, kaempferol, gallotannins, *p*-hydroxybenzoic acid, m-hydroxybenzoic acid, mangiferin, catechins, apigenin, rhamnetin, myricetin, epicatechin, protocatechuic acid, quercetin and anthocyanins (Berardini et al. 2005; Kim et al. 2007; Masibo and He 2008). The gallic acid content in mature green mango (cv. Keaw) was found to be 231 μg/100 g of the fruit, while in the ripe mango of the same variety, the gallic acid content was reported as 397.4 μg/100 g of the fruit (Gorinstein et al. 1999). Rocha Ribeiro et al. (2007) also compared the antioxidant potential of ripe mango pulp amongst four different cultivars (Haden, Tommy Atkins, Palmer and Uba), and significant differences in the bioactive constituents and their levels in the four tested mango varieties were observed. Uba variety showed the highest total phenolics, ascorbic acid and β-carotene contents. Sucrose (19–55.4 g/100 g of freeze-dried mango) was found to be the predominant sugar content of mango, followed by fructose (10–20 g/100 g dried mango) and glucose (2–6.3 g/100 g) (Rumainam et al. 2018). These sugars along with the starch content of the fruit seemed to vary with the stages of maturity (Lechaudel et al. 2005). The cell wall of mango fruit was also shown to contain neutral sugars as well as polysaccharides pectin and hemicelluloses. A decrease in hemicellulose content with ripening was observed, which correlates well with softening of the fruit (Mitcham and McDonald 1992). The presence of minerals, namely, potassium, calcium, sodium, iron, zinc, copper and magnesium, in mango fruit have also been reported (Lechaudel et al. 2005; Lauricella et al. 2017).

Mangiferin, a xanthone was purified from the bark, seed, leaves, peels and pulps of different mango cultivars, and has been known to exhibit various bioactivities. Mangiferin was reported to be present in higher amount in the leaves and seeds than that of the fruit pulp (Saleh and El-Ansari 1975; Luo et al. 2012; Vo et al. 2017). Mango pulp is also rich in various types of vitamins, out of which ascorbic acid represents the highest concentration (Ajila et al. 2007). More than 25 carotenoids are being identified from the mango pulp, and all-trans-β-carotene is reported to be the dominant carotenoid accounting for 48 to 84% of the total carotenoids present in mango pulp. Other carotenoids found in mango include antheraxanthin, zeaxanthin, neoxanthin, luteoxanthin, cryptoxanthin and violaxanthin (Cano and De Ancos 1994; Chen et al. 2004; Ornelas-Paz et al. 2007). The β-carotene content of mango was highest when fully ripened (Singh et al. 2017). Lupeol is another important bioactive triterpene shown to exhibit wide ranges of bioactivities (Prasad et al. 2007). The dietary fibres present in mango peel are mostly made up of uronic acids, lignin, minerals, neutral sugars, protein and lignin (Larrauri et al. 1996).

21.2.5.2 Bioactivities

Bioactive metabolites of mango play a vital role in overcoming various diseases through plethora of mechanisms, including antioxidant activity for combating diseases caused by oxidative stresses. Phenolic compounds, carotenoids and ascorbic acid are the major phytocomponents possessing the antioxidant activity. Further, the antioxidative capacity is correlated to the flavonoid and phenolic content of mango (Rumainam et al. 2018). Ajila et al. (2007) extracted mango peel by using acetone as solvent and reported the presence of carotenoids, polyphenols and anthocyanins, and a satisfactory antioxidant activity was shown by the peel extract. The content of minerals (iron, zinc, manganese and copper) in the mango flesh is low, but these can exert synergistic effect on the free radical scavenging activities of other phytonutrients (Lechaudel et al. 2005; Lauricella et al. 2017).

Mangiferin extracted from different parts of mango plant has been shown to exhibit various bioactivities. Along with gallic acid, it was found to show potential anticancer activity in various cancer cell lines (García-Rivera et al. 2011). The antiproliferative activity was demonstrated on metastatic breast cancer cells, Caco 2 and HT1080 fibrosarcoma cancer cells. The mechanisms by which mangiferin and gallic acid in mango extract exhibit antitumour and anti-inflammatory actions include the inhibition of inflammatory cytokines, i.e., IL-6, IL-8, chemokine receptor type 4 (CXCR4), cyclooxygenase 2 and angiogenesis, while also affecting apoptotic process (García-Rivera et al. 2011). Percival et al. (2006) studied the anticancer activity of mango juice and mango juice extracts in cancer cells by arresting the cell cycle in the G_o/G_1 phase. Mangiferin also shows antidiabetic potential by inhibiting α-glucosidase enzyme (Vo et al. 2017). The hepatoprotective property of lupeol and mango extract has been widely demonstrated through studies and investigations performed over the years (Prasad et al. 2007).

Gallotannins isolated from the kernel of mango were reported to possess antimicrobial property against *E. coli* and other Gram-positive bacteria, mainly due to their capability to form iron complexation (Engels et al. 2009). One more study also demonstrated the capability of mango extracts in endothelial cell migration (Daud et al. 2010). It was shown that mangiferin extracted from mango flesh and peel enhances the migration of endothelial cells, which may be beneficial for improving the diseases related to malformation of new blood vessels.

21.2.6 Guava

Psidium guajava L. (Guava) belongs to the family Myrtaceae, and is the predominant and most important fruit amongst different species of the genus *Psidium*. Guava that is considered native to the American tropics is now widely cultivated throughout the subtropical and tropical areas of the globe (Pommer and Murakami 2009; Correa et al. 2011). Guava fruit is classified botanically as a berry or capsule, and it weighs about 100–250 g, measuring about 5–10 cm in diameter (Singh 2011). Based on the cultivars, the morphology of guava (shape, colour and surface smoothness) may differ. Guava is a popular tropical fruit, and it may be eaten fresh and

made into juice, jams or various other products after the seeds are removed (Marcelin et al. 1993). On ripening, the skin of a guava fruit turns yellow from green and may become softened (Yusof 2003). Guava fruit is considered to be highly nutritious, due to its phytonutrient contents, and widely used in folk medicine around the world (Gutierrez et al. 2008).

21.2.6.1 Bioactive Compounds

The phytochemical constituents and fruit quality of guava can be highly varied depending on various factors like the stage of maturity, season and cultivars (Bashir and Abu-Goukh 2003; Thaipong and Boonprakob 2005). In general, guava fruit is composed of 85% moisture; 0.3% and 0.1% of protein and fats, respectively; 15% total carbohydrates; vitamins; minerals; fibres; and other bioactive phytochemicals (Yusof 2003). The peels and pulp of guava showed a high content of total dietary fibre (TDF) at about 48.00 to 49.00 g/100 g of dry weight, and the insoluble dietary fibre (IDF) in the fruit constitutes more than 97% of this TDF, which contribute towards the potential antioxidant activity of the fruit (Jimenez-Escrig et al. 2001). Between the white-fleshed and pink-flesh guava types, the white-fleshed guavas were reported to contain higher levels of vitamin C, total soluble solids, sugars, titratable acidity and total phenolic compounds (Bashir and Abu-Goukh 2003). Guava is considered to be a rich source of vitamin C. The contents of vitamin C were found to vary between 9 mg/100 g and as high as 560 mg/100 g of the guava fruit. This difference mainly depends on cultivars, growing conditions and other postharvesting factors (Padula and Rodriguez-Amaya 1986). The vitamin C content of guava was found to be three to six times higher than that of oranges (Da Costa et al. 2009). Guava fruit also contains other vitamins, such as niacin, pantothenic acid, thiamin, riboflavin and vitamin A, along with minerals, such as calcium, phosphorus and iron (Rana et al. 2015). Okwu et al. (2003) also suggested that magnesium, sodium, calcium, potassium and phosphorous are the important minerals present in the twigs of guava. The peel of guava was reported to be richer in vitamin C and phenolic compounds than the pulp (Bashir and Abu-Goukh 2003). Citric acid is the predominant organic acid present in guava, found along with other minor organic acids including glycolic, malic and ascorbic acids (Wilson et al. 1982). The main sugar was also found to be fructose, while sucrose and glucose were also present in good amount.

The presence of phytonutrients, such as lycopene, β-carotene, flavonoids and other phenolic compounds in guava is being reported. The fruit pulp of red-fleshed type is rich in carotenoids, especially lycopene and β-carotene (Correa et al. 2011). The types of carotenoids and their concentrations generally determine the colour intensity of fruits. Ripening of the fruit is also associated with higher concentration of total carotenoids (Jain et al. 2003). Mercadante et al. (1999) also identified sixteen carotenoids including lycopene, β-cryptoxanthin, β-carotene, γ-carotene, lutein, phytofluene, cryptoflavin and rubixanthin from Brazilian red-flesh cultivar (IAC-4). Some of the phenolic/flavonoidal compounds identified in guava include quercetin, luteolin, myricetin, kaempferol, apigenin, schottenol ferulate, epicatechin and myricetin (Vargas-Alvarez et al. 2006; Chiari-Andreo et al. 2017; Naseer

et al. 2018). In Mexico, the highest concentrations of flavonoidal compounds in mature fruit were reported to occur in the month of July (Vargas-Alvarez et al. 2006). Other bioactive compounds like leukocyanidins, gallic acid, tannins and sterols are also found to occur at high concentration in different parts of the guava plant (Naseer et al. 2018).

Aromatic profiling of guava fruit had been attempted by different studies. About 34 volatile compounds have been identified from the mature and ripe guava fruits. The important volatile compounds in the mature fruit include 1,8-cineole, (*E*)-2-hexenal and (*E*)-3-hexenal (Chyau et al. 1992). In another study, a total of 51 aromatic compounds were identified in the commercial guava essence and fresh pink guava fruit puree (Jordan et al. 2003). Some of these volatile components include D-limonene, α-pinene, 1,8-cineol, β-caryophyllene, α-copaene, α-selinene, 2-methyl-1-propanol, 1-butanol, hexanol, octanol, furfural, (*Z*)-*β*-ocimene, linalool and α-terpineol.

21.2.6.2 Bioactivities

Different parts of guava have been used for the treatment of various human ailments, including inflammation, diabetes, hypertension, wounds, stomach problems and fever since ancient times (Gutierrez et al. 2008). The ethanolic extract of guava leave was found to exhibit similar antidiarrhoeal activity to that of loperamide when tested against castor oil-induced diarrhoea (COID) model in Wistar rats (Mazumdar et al. 2015). The antidiarrhoeal activity of quercetin, one of the phenolic compounds found in guava fruit was also demonstrated, and it was found to act by interfering with the contraction of ileum (Zhang et al. 2003). Methanolic extract of the fresh leaves of guava exhibited antimicrobial activity against 9 of the 11 microbial strains isolated from wounds including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* spp., *Shigella* spp. and *E. coli* demonstrating its potential in wound healing preparations (Chah et al. 2006). Guava extracts also possess inhibitory activity against *Acne vulgaris* microorganism, *Propionibacterium acnes.* Considering that guava also contain anti-inflammatory components, the extracts of *Psidium guajava* may be highly beneficial in the management of inflammatory acne (Qadan et al. 2005).

Many studies have suggested antidiabetic property of guava extracts in various animal diabetic models (Cheng et al. 2009; Mukhtar et al. 2006). Polyphenolic compounds in guava may be attributed to play a major role in the antidiabetic activity of guava, and also for exerting free radical scavenging activity (Gutierrez et al. 2008). Different flavonoids present in guava act as good sources for dietary fibre that may play an important role in maintaining blood glucose level in diabetic conditions (Cheng et al. 2009; Ojewole 2006). Although less potent than the available antidiabetic drugs, chlorpropamide and metformin, oral administration of guava juice has the ability to reduce blood glucose level (hypoglycaemic effect) in both maturity-onset diabetic and healthy volunteers (Cheng and Yang 1983).

Extracts of guava leaf have been employed for treating different inflammatory diseases such as rheumatism. The leaf extract was found to possess anti-inflammatory and analgesic properties which could help in the management of painful arthritic

and other inflammatory conditions (Ojewole 2006). The essential oils present in guava leaves also exert antiproliferative activity, where monoterpenes identified in the essential oil were attributed to play a prominent role (Manosroi et al. 2006).

21.2.7 Pomegranate

Pomegranate (*Punica granatum*), a fruit-bearing small tree plant, belongs to the family Punicaceae. Being native to Asian region, mostly Iran, it is found occurring in many regions of Afghanistan to the Himalayas in North India (Jurenka 2008; Sadeghipour et al. 2014). However, it is now naturalized throughout the Mediterranean countries and also cultivated in Southeast Asia and the United States, along with some other countries. The pomegranate fruit may be consumed fresh or used to make various food products including canned juice and beverages, jams, jelly and paste, flavours, and colours (Viuda-Martos et al. 2010). The medicinal properties of pomegranate fruit had been described in Ayurvedic medicine and were considered 'a pharmacy unto itself' (Jurenka 2008). Both the edible and nonedible parts of pomegranate fruit contain bioactive compounds that are essential for promoting health (Viuda-Martos et al. 2010; Akhtar et al. 2015).

21.2.7.1 Bioactive Compounds

The peels, juice and seeds of pomegranate contain various bioactive compounds that are known to possess innumerable health benefits. They are a rich source of polyphenolic compounds, including flavonoids and hydrolysable tannins, which are responsible for 92% of their antioxidant activity (Abid et al. 2017). The major phenolic compound reported in the fruit of pomegranate includes caffeic acid, catechin, chlorogenic acid, o- and p-coumaric acids, ellagic acid, ferulic acid, gallic acid, phloridzin, protocatechuic acid and quercetin (Poyrazoglu et al. 2002; Mousavinejad et al. 2009; Nuncio-Jauregui et al. 2015; Abid et al. 2017). The amount of phenolic acid and total phenolic contents in pomegranate fruit may vary amongst different cultivars (Mousavinejad et al. 2009). Citric acid was reported as the predominant organic acid present in the fruit along with other acids, such as L-malic acid, oxalic acid, quinic acid, tartaric acid and succinic acid (Poyrazoglu et al. 2002). Pomegranate has been considered as one of the major anthocyanin-rich fruits (Jaiswal et al. 2010; Hasnaoui et al. 2011). Delphinidin 3,5-diglucoside (372–5301 mg/L) and cyanidin 3,5-diglucoside (242–2361 mg/L) are the major anthocyanins in pomegranate (Mousavinejad et al. 2009). Volatile compounds are the major constituents that influence consumer's liking of pomegranate juices (Calın-Sanchez et al. 2011). Volatile compounds abundantly found in the fruit include 3-carene, hexanal, trans-2-hexenal, cis-3-hexenol, limonene, α-terpinene and α-terpineol (Calın-Sanchez et al. 2011; Melgarejo et al. 2011). Different levels of both macro- (P, K, N, Mg, Ca and Na) and micronutrients (Zn, Cu, Mn, Fe and B) were found in pomegranate fruits, however, the levels of which may change with the growth and development of the fruit (Mirdehghan and Rahemi 2007). Fructose and glucose were the two major sugars detected in pomegranate juices (Hasnaoui et al. 2011).

21.2.7.2 Bioactivities

The medicinal values of pomegranate have been realized for decades, and several excellent reviews on their therapeutic applications are widely available (Jurenka 2008; Viuda-Martos et al. 2010; Akhtar et al. 2015; Rahmani et al. 2017). The antioxidant compounds present in the fruit are known to play a significant role in protecting the body from diseases caused by oxidative stresses including cancer, cardiovascular disorders and diabetes (Ignarro et al. 2006; Jurenka 2008; Johanningsmeier and Harris 2011). The extracts of pomegranate peel, leaf, seed and whole fruits have antimicrobial properties against various pathogenic bacteria and fungi, including *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhimurium* and *Aspergillus niger* (Dahham et al. 2010; Ismail et al. 2016; Alexandre et al. 2019). The peel extract was found to contain punicalagin, which was shown to be very effective against Gram-positive bacteria than Gram-negative bacteria, while showing no inhibitory activity against the beneficial lactic acid bacteria (Alexandre et al. 2019). In another report, the antimicrobial activity of pomegranate peel extracts was affected by the cultivars type, and ellagic acid content was reported to have a significant influence on the antimicrobial activity of the peel extract (Rosas-Burgos et al. 2017).

The pomegranate juice was shown to exhibit antiproliferative activity against different cancer cells including human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620) and prostate (RWPE-1, 22Rv1) tumour cells. Its antiproliferative activity was superior to its purified polyphenols, such as punicalagin and ellagic acid (Seeram et al. 2005). Apart from its antioxidant, anti-inflammatory and lipid-lowering activities, pomegranate juice was also found to boost the well-being and vigour of pancreatic islets of Langerhans in diabetic animal model, which suggested that pomegranate juice would be highly beneficial in the management of diabetes and its associated conditions, such as preventing oxidation, inflammation and accumulation of unhealthy fats (Taheri-Rouhi et al. 2017).

21.2.8 Litchi

Litchi or lychee (*Litchi chinensis* Sonn.) is an evergreen tree found in subtropical to tropical region. It belongs to the family, Sapindaceae. Litchi is believed to originate from China, where it has been cultivated for thousands of years (Mitra and Pathak 2010). The fruits are small and vary in shapes from round to egg or heart shape, and the colour may also vary depending on the cultivars (Menzel 2003). The edible part of the fruit is known as aril, and the seed constitutes a significant portion of the fruit accounting for 10–20% of the fruit weight (Menzel 2003; Yang et al. 2011). Litchi and other products made from the fruits have been widely accepted and consumed worldwide, which enhance its commercial value (Jiang et al. 2013). Southeast Asian countries including China, India, Vietnam, Bangladesh and Thailand contribute about 95% of the world's litchi production, and India produces about 585,300 metric tonnes of litchi fruit annually (Huang et al. 2005; Kisku et al. 2017). Being considered healthy fruit, however, recent studies have raised concern over the

consumption of litchi and some other Sapindaceae fruits under certain conditions due to their possible role in causing hypoglycaemic encephalopathy amongst malnourished children as a result of their phytochemical components (Shrivastava et al. 2017; Spencer and Palmer 2017).

21.2.8.1 Bioactive Compounds

The seed and pericarp of litchi are rich in bioactive phytochemicals, including flavonoids and other phenolic compounds. A 100 g of fresh litchi fruit contains 84 g of water, 15 g of carbohydrates, 32 mg of phosphorous, 15 mg of vitamin C and traces of others including thiamine, niacin, riboflavin, iron, calcium, protein and fat (Huang et al. 2005). The major anthocyanin identified in litchi is cyanidin-3-rutinoside, while the occurrence of malvidin-3-glucoside and cyanidin-3-glucoside is also detected (Lee and Wicker 1991). The phenolic compounds identified in the pulp of litchi fruit include 3,4-dihydroxybenzoic acid, catechin, caffeic acid, 4-methylcatechol, chlorogenic acid, ferulic acid, epicatechin, gallic acid, quercetin 3-rut-7-rha, syringic acid and vanillic acid (Li and Jiang 2012; Zhang et al. 2013; Su et al. 2014a, b). The seed of the fruit was also found to contain compounds such as glycosides of flavonoids kaempferol, taxifolin, rutinosides and phlorizin along with coumaric acid, protocatechuic acid, narirutin, naringin, litchiol A and B and many others (Xu et al. 2010, 2011). Predominant sugars in litchi include glucose, fructose and sucrose. Malic, tartaric, citric and ascorbic acids were the organic acids reported from litchi fruit, where malic acid was found to be the major organic acid (Wang et al. 2006). Thirteen different minerals have been detected in the fruit pulp including Ca, Mg, Zn, K and Na (Cabral et al. 2014).

In 1962, the isolation of a hypoglycine A analogue, α-(methylenecyclopropyl) glycine (MCPG), was reported from the seeds of litchi (Gray and Fowden 1962). MCPG was shown to exhibit strong hypoglycaemic activity and implicated to cause acute encephalopathy in animal models (Melde et al. 1991; Isenberg et al. 2015). It has been reported that the existence of MCPG also observed in litchi pulp (Das et al. 2015).

Apart from vitamin C, the fruit pulp of litchi was also reported to contain vitamin E, namely, γ-tocopherol, α-tocotrienol, α-tocopherol and γ-tocotrienol. However, both beta-carotenes and lycopenes were not detected in both the Brazilian and Thai varieties, and the amount of vitamin E present was found to depend on the cultivar types (Charoensiri et al. 2009; Cabral et al. 2014). Tocotrienol, a member of vitamin E family, was also reported from the leaves of litchi (Lin et al. 2015). Litchi seeds are important source of cyclopropanoic acid, and the ethanol extract of seeds exhibited antiproliferative activity comparable to that of cisplatin (Yang et al. 2011).

21.2.8.2 Bioactivities

The pulps of litchi fruits contain phenolic compounds, and are recognized for higher antioxidant activity. Various in vitro models including DPPH, oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP) and cellular antioxidant activity in HepG2 cells were used to prove the same (Jiang et al. 2013; Zhang et al. 2013; Su et al. 2014a, b). The hepatoprotective property of the fruit pulp

restraint stress-induced liver injury in animal model was attributed to its phenolic components (Su et al. 2016). The fruit pulp extracts of different litchi cultivars exhibit hepatoprotective property against CCl_4-induced hepatotoxicity in animal model through anti-lipid peroxidation and anti-apoptotic activities (Bhoopat et al. 2011). The antioxidant possessions of the fruit pulp were, however, found to vary depending on the litchi fruit cultivars (Bhoopat et al. 2011; Zhang et al. 2013).

The fruit pericarp of litchi contains several proteins and polysaccharides. Studies have found the antioxidant, antiproliferative and immunomodulatory activities of these polysaccharide fractions isolated from litchi fruit pulps (Hu et al. 2011, 2015; Huang et al. 2014, 2015, 2016). The free radical scavenging activity of these natural polysaccharide fractions has been compared to vitamin C, and it was found that if 100% bioavailable, the antioxidant effect of consuming of litchi pulp tissue (roughly 200 g) might result in a antioxidative effect that is similar to the suggested every day dosage of vitamin C (Hu et al. 2011). It has been stipulated that the use of litchi in Chinese traditional medicine may be due to the antioxidative potential of the polysaccharide fractions in the fruit pulp (Hu et al. 2015). The rationale against the use of dried litchi pulp in Chinese traditional medicine had also been investigated, and found that rather than the fresh pulp polysaccharides, the dried pulp polysaccharide fractions exhibited higher cytotoxicity against HepG2, HeLa and A549 cells and also recorded with immunomodulatory activity (Huang et al. 2014). The condition for isolation of the polysaccharide fractions from the pulp is critical as it influences the monosaccharide composition as well as the other components in the polysaccharide fractions, which in turn determine the antioxidant and antiproliferative activity of the polysaccharides (Huang et al. 2015).

Apart from being exhibiting a strong antioxidant activity, the bioactive compounds in the seed extracts showed a wide ranging activities, including anticancer, hypoglycaemic and antibacterial properties (Xu et al. 2010; Wang et al. 2011; Bhat and Al-daihan 2014; Lv et al. 2014; Man et al. 2016; Guo et al. 2017). Due to its ability to reduce the levels of serum cholesterol and low-density lipoproteins in blood, the seed oil of litchi was also considered to possess beneficial effect against cardiovascular diseases (Yang et al. 2011).

21.2.9 Sapota/Sapodilla

Sapota, also known as sapodilla (*Manilkara zapota* (L.) P. Royen is native to tropical America, and belongs to the family, Sapotaceae. It is believed to originate from Southern Mexico or Central America (Britto and Narain 2002). *Manilkara zapota* has several synonyms, including *Achras sapota* L., *A. zapota* L., *A. sapatilla* J. Paul & W. Arnold, *A. zapotilla* (Jacq.) Nutt., *A. mammosa* L. nom. Illeg., *M. achras* (Mill.) Fosberg, *M. zapotilla* (Jacq.) Gilly, *Sapota zapotilla* (Jacq.) Coville ex Safford and *S. achras* Mill. (Lim 2013). Sapota is one of the major fruit crops in tropical countries, including India, Venezuela and Mexico (Kulkarni et al. 2007). The mature fruit of sapota is globose, ellipsoid or ovoid in shape and is delicious and considered to be highly nutritious (Lim 2013; Siddiqui et al. 2014).

21.2.9.1 Bioactive Compounds

The fruit pulp, peels, seeds and leaves of sapota were all shown to contain various bioactive phytochemicals, and their health benefits have been widely explored. The polyphenolic contents in the fruit pulp of Indian sapodilla include gallic acid, catechin and quercetin, while the peel contains gallic acid, catechin, quercetin and kaempferol (Singh et al. 2016a). The amount of polyphenolic contents in the peels was significantly higher than that of the fruit pulp. Methanolic extracts of the fruit collected from Florida in the United States were shown to contain catechin, epicatechin, dihydromyricetin, gallic acid, gallocatechin, 4-O-galloylchlorogenic, methyl chlorogenate, methyl 4-O-galloylchlorogenate, myricitrin and quercitrin (Ma et al. 2003). For every 100 g of the fruit, sapodilla (Mexican variety) was reported to contain 21.43 mg of vitamin C, 1.16 g of anthocyanins, 15.35 mg of phenolic compounds (gallic acid equivalents), 0.18 mg of flavonoids (quercetin equivalents) and 1.69 mg of carotenoids (β-carotene equivalents) (Moo-Huchin et al. 2014). The phenolic compound in Florida-grown sapodilla was mainly the catechin conjugate (Mahattanatawee et al. 2006). Apart from the phenolics, carotenoids and vitamin C, the fruit juice of sapodilla also contains minerals such as iron, copper, zinc, calcium and potassium (Kulkarni et al. 2007). Bioactives such as β-amyrin-3-(3′-dimethyl) butyrate, lupeol-3-acetate and cryptochlorogenic acid have also been reported from the fruit extracts (Fayek et al. 2013). The major sugars identified in the fruit of sapodilla were sucrose, glucose and fructose with fractions of starch (Selvaraj and Pal 1984). Like other fruits, the phytochemical contents of sapodilla also change as the fruit progressed from mature, to half-ripe, to full-ripe stages. On approaching the maturation, there was an increase in the level of reducing sugars in the fruit; however, tannin levels reduced significantly, and the vitamin C content also reduced to traces on ripening (Britto and Narain 2002). The major organic acids in mature sapodilla fruits were reported to be malic acid, citric acid and tartaric acid (Sumathi and Shivashankar 2017).

The leaves of sapota were also reported to contain several bioactive compounds. The compounds isolated from the leaves include oleanolic acid, caffeic acid, lupeol acetate, myricetin-3-O-α-L-rhamnoside and apigenin-7-O-α-L-rhamnoside (Fayek et al. 2012). About 69 aroma volatiles in the sapodilla fruit were identified using gas chromatography-mass spectrometry (GC/MS) analysis; the major components of the fruit volatiles were methanethiol, 3-hydroxy-2-butanone, hexadecanoic acid, ethyl acetate and isoamyl alcohol (Pino et al. 2003). The seeds contain a diverse classes of phytochemicals, such as sapotin, saponin, and sapotinine. The seed coat of sapodilla fruit extract contains gallic acid, chlorogenic acid, and quercetin (Kanlayavattanakul and Lourith 2011).

21.2.9.2 Bioactivities

Ripe sapodilla fruits provide abundant supply of sugars, protein, carotenoids, phenolics and ascorbic acid. The young fruits, leaves and seeds were considered medicinal in Indian traditional medicine and used to treat diarrhoea and pulmonary problems, while crushed seeds were used as diuretics to expel kidney or bladder stones (Kulkarni et al. 2007). The antioxidant activity of the fruit can be largely credited to the polyphenols, such as catechin and gallocatechin (Lim 2013), and this multiple radical scavenging potential also correlates well to the

presence of phenolics, ascorbic acid and polyphenols (Kulkarni et al. 2007). Fruit extracts of sapota was also shown to possess high anticollagenase and anti-elastase activities with moderate antioxidant effect which indicate their anti-ageing effect (Pientaweeratch et al. 2016).

A range of anticancer activities have been shown by phenolic compounds from sapota fruit. For example, cytotoxicity in the human colon cancer cells (HCT-116 and SW-480) was shown by methyl 4-*O*-galloylchlorogenate, which was isolated from sapota fruit (Ma et al. 2003). The methanolic extract of sapodilla fruit also induces apoptosis in pre-B cell leukaemia (NALM6) and chronic myelogenous leukaemia (K562) cells. Further, treating tumour-bearing mice with the extract was also shown to prolong the life span of the animals by three times compared to the untreated mice (Srivastava et al. 2014). Fayek et al. (2012) showed the hypocholesterolaemic and antihyperglycaemic activities of the leave extracts of sapota and also confirmed the antioxidant actions of both the aqueous and alcoholic extract of the leaves. The leave extract also demonstrated antimicrobial property against 19 pathogenic microbes including *P. aeruginosa*, *S. aureus*, *B. subtilis*, *S. typhimurium*, and *K. Pneumonia* (Nair and Chanda 2008).

21.3 Conclusions and Future Perspectives

India is one of the major producers of fruits in the world. The major fruits include mango, banana, different citrus fruits, guava, grape, pineapple and apple. The consumption of vegetables and fruits has been central to the Indian diet system, since ancient times. Both vegetables and fruits are a rich source of dietetic fibres, vitamins, macronutrients, minerals and other phytochemicals. In this chapter, various tropical fruits and their phytochemical contents have been discussed along with their bioactivities. Various bioactive compounds reported from Indian fruits included carotenoids, vitamins, catechin, epicatechin, gallic acid, lignin, tannins, anthocyanins, lutein, α-carotene, β-carotene, auroxanthin, violaxanthin, neoxanthin, α-cryptoxanthin, β-cryptoxanthin, myricetin, catechin, epicatechin, quercetin, kaempferol, sitosterols, β-sitosterol, stigmasterol and campesterol. Their potential health benefits have largely been explored. These phytoconstituents are reported to display several therapeutic properties, such as antioxidative, anti-inflammatory, antimicrobial, anticancer, wound-healing and antidiabetic properties to name a few. Also, Indian fruits were scientifically proven to regulate immune responses and manage diarrhoea in infants and in the treatment of osteoarthritis, cataracts, etc. Most of the tropical fruits are abundant and nutritious with excellent taste and flavours. The growing conditions, the parts of the plant and their stage of maturity seemed to influence the types of bioactive contents and their concentrations.

The growing awareness about the potential health benefits of fruits including their chemopreventive and medicinal properties makes tropical fruits promising and cost-effective functional foods. A policy to promote availability and access to vegetables and fruits is necessary to increase their consumption amongst the people. Increasing awareness on the health efficacies of fruit consumption and the promotion of research to explore the nutritional properties of more Indian tropical fruits would help the development of a balanced and healthier diet to support human life.

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Citrus Flavonoids in Preventing Cardiovascular Diseases

22

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Contents

Abstract

Citrus, belonging to the family of *Rutaceae* is a genus of the flowering plants and shrubs. They originated in the tropical and subtropical regions of Southeast Asia. *Citrus* fruits are highly produced in China, Brazil, USA, India, Mexico, and Spain. Among *Citrus* species, sweet oranges (*Citrus sinensis*) are the most widely spread and valued throughout the world. *Citrus* fruits are rich in

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flavonoids, mainly hesperidin and naringin that are known to have benefit in the avoidance of long-term diseases. Based on the in vivo and epidemiological studies done previously, the potential biological properties of *Citrus* flavonoids in *Citrus* fruits are beneficial for the prevention of cardiovascular diseases (CVDs) by acting as antihypertensive, anti-hypercholesterolemia, and antidiabetic. However, the mechanisms of action are still uncertain and not clearly defined. *Citrus* contains some phytochemicals that can give beneficial effects to human's health through several biological properties. Thus, *Citrus* fruits can be a new discovery of natural prevention of chronic diseases around the globe with regard to their high production each year. This chapter provides a comprehensive information on the phytochemical constituents found in *Citrus* fruits and their biological properties in preventing cardiovascular diseases.

Keywords
Citrus · Biological properties · Phytochemical · Hesperidin · Naringin

22.1 Introduction

Citrus is a genus of the flowering plants and shrubs, and belongs to the family of *Rutaceae*, which is believed as a local and mother plant to the subtropical and tropical regions of Asia. It is seen growing in certain areas of Southeast Asian countries, including China, India, and the Malay Archipelago. Yet, the true history about the beginning of how *Citrus* fruit was discovered is brimful of disputation. In Romance loanword, *Citrus* is known as *agrumes*, which means sour fruits (Liu et al. 2012). Due to its enormous benefits and high demand, *Citrus* fruits have become the world's most important fruit tree crop since the last decades of the twentieth century with more than 105 million tons of all types of *Citrus* fruits produced every year (Ladaniya 2008). *Citrus* fruits is composed of many and unclear natural species, but among them, there are few species that are commercially important worldwide as it grants a lot of positive effects to human consumptions. It includes sweet orange (*C. sinensis*) that covers about 60% of total production, tangerines (*C. reticulata*), lime (*C. aurantifolia*), lemon (*C. limonum*), and grapefruit (*C. paradisi*) (Okwu 2008). The growth of *Citrus* fruits covers more than 140 countries throughout the world; however, most of the crop trees grow in the subtropical and tropical areas of the world as its cultivation and production mainly concentrate in the regions of the Northern Hemisphere (Ladaniya 2008). Due to the high request, the global production of *Citrus* fruits grows expeditiously as reported that the total world production of the *Citrus* fruits increases rapidly starting from 2007 to 2014 in which China, Brazil, USA, India, Mexico, and Spain are acknowledged as the world's supreme countries in yielding of *Citrus* (FAO 2015).

The evergreen *Citrus* trees produce fruits with a variety of forms and sizes, and also full of fragrance, flavor, and juice which differ from each species (Okwu 2008).

Citrus fruit has a rough, vigorous, and bright color that ranges from green to yellow skin or rind known as epicarp which acts as the outermost skin of fruits that covers and protects them; the flavedo is the subepidermal layer that contains oil sacs which produce aromatic oils, the thick and squashy layer subservient to the flavedo is known as albedo, and vascular bundles are a network of thin thread along the flesh. Apart from that, the internal part of the fruits consists of segments which are covered by the septum and is filled by small and compacted packed sacs that contain juice and seeds. The juice in albedo consists of citric acid and other acids, oils, and sugars that are responsible for the distinction flavor of the fruits (Liu et al. 2012). The constituents of *Citrus* fruits are magnificently beneficial as it contains a lot of phytonutrients and phytochemicals, mainly flavanones, which primarily occur in the albedo part of fruits as shown in Fig. 22.1.

Cardiovascular disease (CVD) is the major leading cause of death among adults in the USA (Go et al. 2014) and remains the first cause of death globally (https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)). In 2015, about 17.7 million people died from CVD which represent 31% from the total worldwide deaths, and among these deaths, an approximate of 7.4 million were due to coronary heart disease, and 6.7 million were due to stroke (https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)). CVD is a group of disorders that is related to the heart and blood vessels (Mendis et al. 2011). Common CVD includes coronary artery disease and cerebrovascular disease (stroke) due to the problems in supplying blood to the heart and brain. The occurrences of CVD among individuals are due to several risk factors, either behavioral or metabolic (biomedical) risk factors. Behavioral risk factors include unhealthy diet, tobacco smoking, severe alcohol intake, and sedentary lifestyle. In addition, metabolic risk factors such as atherosclerosis, hypertension, hyperlipidemia, diabetes mellitus, and obesity contribute more in developing CVD (Mendis et al. 2011). Among all these risk factors, the three major risk factors for global disease are arterial hypertension, cigarette smoking (including secondhand smoke), and household air pollution from solid fuels that were reported in 2010 (Lim et al. 2012).

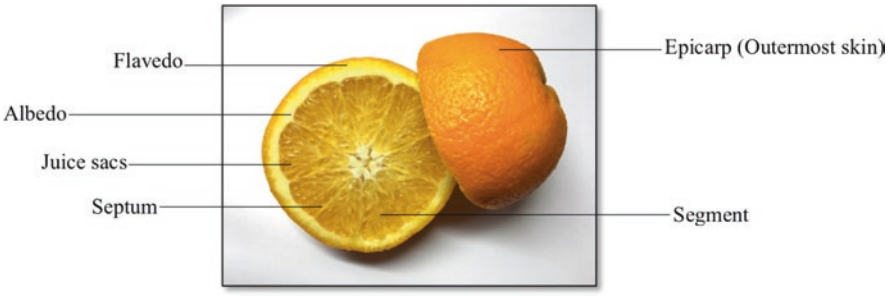


Fig. 22.1 Structure of citrus fruits

A large percentage of CVD is preventable by the reduction of behavioral risk factors (https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)). Management of CVD worldwide can be either through non-pharmacological or pharmacological approaches. Lifestyle modifications are the best management above all. However, for the pharmacological approaches, CVD can be managed by controlling the risk factors such as administration of antihypertensive agents, cholesterol-lowering agents, and antidiabetic agents. Some of the previous studies found that eating of fresh and healthy vegetables and fruits can lower the risk of development of CVD in most of individuals, due to the potential health-promoting constituents found in vegetables and fruits (Liu 2003).

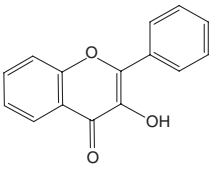
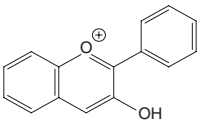
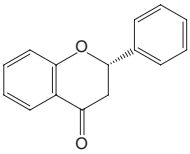
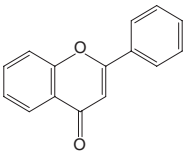
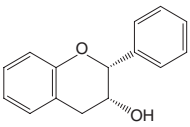
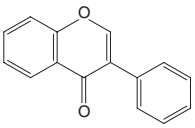
Previously, many studies have been done by the researchers about *Citrus* as to discover the benefits of their polyphenolic compound. The main active compounds found in *Citrus* are naringin and hesperidin which have various biological properties such as blood pressure-lowering effect, reduction of lipid, insulin sensitizing, and antioxidative and anti-inflammatory properties which confer their effect in inhibiting atherosclerosis. Hence, this review paper is done mainly to focus on the potential biological properties of *Citrus* flavanones in cardioprotection by reducing the effect of risk factors on the evolution of cardiovascular diseases (CVDs). This chapter was assisted from few previous findings of in vivo and epidemiological studies obtained from published journals, articles, and books ranging from year 1990 to 2017 which are available from some databases including PubMed, ScienceDirect, Elsevier, and Google Scholar.

22.2 Phytochemical Constituents

Unhealthy diet and low intake of polyphenols are the main factors that are conducive to the cardiovascular disease (CVD) events. CVD has several causes as majority of the CVD events are due to the pathophysiology disorders, such as hypertension, hyperglycemia, and hyperlipidemia which may lead to the development of atherosclerosis, a disease in which plaque builds up in the arteries (Habauzit and Morand 2012). The consumption of plant-based food such as fruits, vegetables, nuts, whole grains, and plant-based beverages shows a promising effect on the reduction of incidence of CVD (Dauchet et al. 2005).

During the last 10 years, most of the study were focusing on the polyphenols, a large family of phytochemicals that acts as antioxidant, which is largely available in our diet (Scalbert et al. 2005). Polyphenols are mainly found in the plants as secondary metabolites characterized by a basic structure having an aromatic ring with one or more hydroxyl groups which are divided into simple phenol, flavonoids, and non-flavonoids (Chanet et al. 2012). An estimated daily intake of total polyphenols was about 1.2 g/day which constitutes 40% flavonoids and 60% phenolic acids (Pérez-Jiménez et al. 2011). Flavonoids are the second group of polyphenols that are largely present in human diet with the structure consisting of two or more aromatic rings connected to three carbons.

Table 22.1 Subclasses of flavonoids with their dietary flavonoids and common food sources

Flavonoids subclasses	Chemical structure	Dietary flavonoids	Food sources
Flavonols		Quercetin, kaempferol, myricetin, isorhamnetin	Yellow onions, scallions, kale, broccoli, apples, berries, teas
Anthocyanidins		Cyanidin, delphinidin, malvidin, petunidin, peonidin, pelargonidin	Red, blue, and purple berries; red and purple grapes; red wine
Flavanones		Hesperetin, naringenin, eriodictyol	Citrus fruits and juices, examples: oranges, grapefruits, lemons
Flavones		Apigenin, luteolin	Parsley, thyme, celery, hot peppers
Flavan-3-ols		**Monomers:** Catechin, epigallocatechin **Polymers:** Proanthocyanidins	Green teas, chocolate, grapes, berries, apples Chocolate, grapes, berries, apples
Isoflavones		Daidzein, genistein, glycitein	Soybeans, soy foods, legumes

From various types of plants, *Citrus* plants are a good source of flavonoids. Flavonoids can be divided into several subclasses, which are flavanones, flavanols, flavonols, anthocyanidins, flavones, and chalcones (Andriantsitohaina et al. 2012). The list of flavonoid subclasses and its chemical structure, dietary flavonoids, and food sources is in Table 22.1. However, among all these subclasses, flavanones account for approximately 95% of the total flavonoids found in *Citrus* fruits (Peterson et al. 2006a, b). In addition, the focus on flavanones is particularly relevant, because of its high content in *Citrus* fruits and the worldwide consumption of *Citrus* fruits has markedly increased recently.

The main flavanones found in *Citrus* fruits are naringin which is mainly found in grapefruits and sour oranges and hesperidin mainly present in sweet oranges,

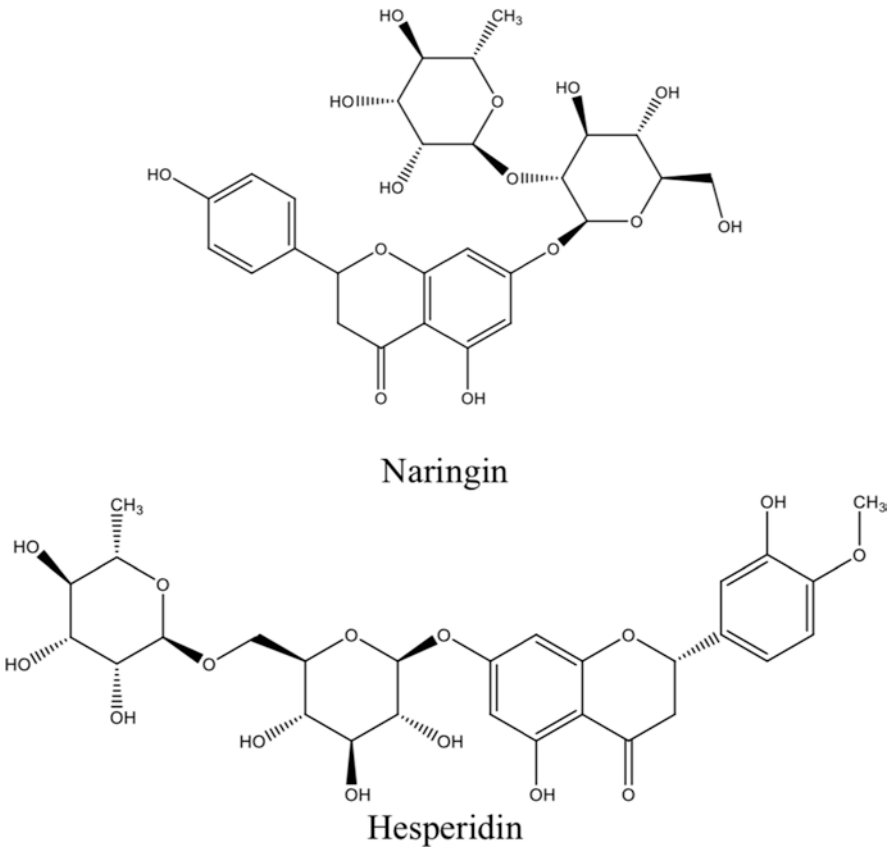


Fig. 22.2 Chemical structure of naringenin and hesperidin

mandarins, and lemons which are generally hydrolyzed by the colon microflora to the corresponding aglycones naringenin and hesperetin, respectively (Fig. 22.2). The various parts of the *Citrus* fruits are individually important as they contain different amount of flavanones. The contents of flavanones in the original fruits are higher compared to juice because abundance of flavanones is found in the albedo (the spongy layer below the subepidermal layer) and the septa rather than the juice vesicles or pulp (Tomas-Barberan et al. 2000).

22.3 Biological Properties of *Citrus* Flavonoids

Daily intake of healthy diet can consequently prevent an individual from developing any diseases and harmful effects to their body. Based on current studies, the researchers also suggest that intake of drinks or foods containing high concentration of flavonoids plays a significant role in boosting the endothelial function as well as

anti-inflammation which further reduce the cardiovascular disease (Habauzit and Morand 2012).

22.3.1 Antihypertensive

Hypertension or high blood pressure is one of the risk factors that can enhance the spread of cardiovascular disease (CVD). The reduction of blood pressure has become the major important parameter in prevention of the CVD event. The consumption of flavonoid-rich food confers a beneficial effect on the blood pressure reduction. Grapefruit juice consumption in hypertensive patients exerts a helpful effect on the blood pressure (Reshef et al. 2005). In addition, one study conducted in 2011 claim that drinking of orange juice and purified hesperidin after four weeks has significantly decrease the diastolic blood pressure (DBP) in healthy, middle-aged, moderately overweight men patients (Morand et al. 2011). This signifies that hesperidin could causally be linked to the helpful effect of orange juice as it represents about 90% of total flavanones in orange fruit.

The possible mechanism by which the flavonoid-rich foods lower the blood pressure is due to long-term extend in the release of nitric oxide (NO) by vascular endothelium (Grassi et al. 2009; Rizza et al. 2011). Therefore, this will increase the blood flow and oxygen transfer to all parts of the body. Apart from that, in 2006, a study has been conducted to evaluate the activity of angiotensin-converting enzyme (ACE) in the presence of flavonol-rich food, and they found that flavonoids could reduce the ACE activity which consequently is responsible for the blood pressure-lowering effect. The finding was then being supported by the present study which focuses more on the structural activity of flavonoids with ACE activity inhibitory effect, where they found that the combination of substructures on flavonoid skeleton is responsible in the ACE inhibition activity (Actis-Goretta et al. 2006; Guerrero and Castillo 2012).

Hesperidin increases the cellular level of phosphorylated AMPK and Akt which then regulate the activity of eNOS resulting in increased production of NO. Besides, orange juice which is also recognized as the main source of hesperidin could prevent the postprandial regeneration of reactive oxygen species (ROS). As it can reduce the oxidative stress, it may increase the NO bioavailability which causes vasodilatation and eventually improves the endothelial function. In addition, NO also can diffuse into the surface of endothelial cells which may prevent the platelet adhesion and aggregation as well as adhesion of monocytes which can further improve the endothelial function (Andriantsitohaina et al. 2012).

Apart from that, prostaglandin also plays an important role in reducing the blood pressure by giving a vasodilatation effect through acetylcholine-mediated vasodilatation by acting on the cyclooxygenase (COX) pathway through the production of prostacyclin, a potent endogenous vasodilator and inhibitor of platelet aggregation (Turner et al. 2008). This hypothesis has been supported from the previous study which shows the ability of the dietary flavonoids to increase the production of prostacyclin (Hermenegildo et al. 2005). The effect of prostacyclin is essentially

opposite with the thromboxane A2, a potent vasoconstrictor which also promotes the aggregation of platelet in blood vessel. It is proposed that the balance between both prostacyclin and thromboxane A2 is tremendously important as they regulate the vascular tone and platelet aggregation (Majed and Khalil 2012).

In addition, the ability of flavonoids to activate endothelial NO synthase is likely the underlying mechanism which may be helpful in improving the endothelial function and therefore affecting the blood pressure. This statement was supported by the study conducted by Morand et al. (2011); both orange juice and pure hesperidin showed positive modifications in the microvascular epithelial function, suggesting that hesperidin could at least partially explain the effect of orange juice. However, the effect of orange juice on microvascular function is greater than the pure hesperidin as it contains vitamin C, which may further contribute to the preservation of healthy vasculature through the modulation of prostacyclin activity and NO bioactivity (Hornig 2002). Hence, from all these previous studies, it can be concluded that the changes in the microvascular function and stability are profoundly related to the plasma concentration of flavonoids.

22.3.2 Anti-hypercholesterolemia

Excessive generation of reactive oxygen species (ROS), known as a state of oxidative stress, may contribute to the vascular pathology which leads to various consequences including CVD disease. Oxidative stress is able to nurture the oxidation of low-density lipoprotein (LDL). LDL is generally known as "bad" cholesterol as it can collect in the walls of blood vessels and cause blockage. An abundance amount of oxidized LDL in blood will be uptake by macrophages and transforms them into foam cells, which are the major components that contribute to the atherosclerotic plaque, the earliest identifiable evidence of atherosclerosis (Cobbold et al. 2002). The formation of the plaques on the vessel walls will restrict the blood flow to various organs especially the vital organs such as the heart and lungs, consequently leading to heart attack and stroke.

Recently, many epidemiological studies propose that the consistent consumption of fruits and vegetables especially *Citrus* fruits may improve healthy life especially in those who are suffering from CVD events as the *Citrus* flavonoids can reduce coronary heart disease (CHD) by preventing the LDL from being oxidized and forming a fatty streak (Djoussé et al. 2004; Dauchet et al. 2005). The mechanisms by which the flavonoids inhibit LDL oxidation are uncertain, but there are several possible proposed mechanisms which may contribute to the inhibition of LDL from being oxidized. Firstly, the flavonoids are said to reduce the generation and release of free radicals in macrophages or protect the α-tocopherol in LDL from being oxidized by the harmful free radicals. Secondly, flavonoids may also terminate the chain of lipid peroxidation by increasing the regeneration of active α-tocopherol by donating its own hydroxyl (OH) atom to α-tocopheryl. Thirdly, flavonoids prevent the LDL oxidation by isolating the metal ions, such as iron and

copper, and therefore declining the endangered free radicals from showing its oxidizing effects (de Whalley et al. 1990).

Moreover, Wilcox et al. (2001) found that *Citrus* flavonoids naringenin and hesperetin are able to modulate apolipoprotein B (ApoB) secretion and therefore reduce the cellular cholesteryl ester mass as it decreases in the cholesterol esterification process. This vastly reduces the capacity of lipoproteins, allowing less efficient cholesterol transport in bloodstream. The decrease in the conversion of free cholesterol is mainly due to the decrease in two enzymes, acyl coenzyme A and cholesterol acyltransferase types 1 and 2 (ACAT1 and ACAT2) which are responsible for cholesteryl ester formation in the tissue. Additionally, naringenin and hesperetin also inhibit both activity and expression of microsomal triglyceride transfer protein (MTP), a lipid transfer protein resulting in decrease in the ApoB secretion. Apart from that, an enhance expression of LDL receptor on the cells may increase in the amount of LDL intake, thus reducing its concentration in the plasma.

In summary, *Citrus* flavonoids may act as protective agents against cardiovascular diseases. Their action is influenced by several vital mechanisms such as preventing the oxidation of LDL, reducing both the activity of ACAT1 and ACAT2, and lastly inhibiting MTP activity. All of these mechanisms are highly beneficial and explain the hypocholesterolemic properties of *Citrus* flavonoids. However, there is some limitations from few previous studies which claim that pure naringenin and hesperidin are not highly effective in reducing serum cholesterol in moderately hypercholesterolemic men and women (Demonty et al. 2010).

22.3.3 Antidiabetic

Hyperglycemia and hyperlipidemia are the common features for diabetes mellitus (DM). Chronic hyperglycemic conditions may increase the concentration of triglycerides (TGs), cholesterol, free fatty acids (FFAs), and phospholipids (PLs) in plasma resulting in hyperlipidemia. Persistent elevation of blood glucose level due to deficiency of insulin may contribute to the onset of DM, a well-known chronic disease worldwide. People with diabetes are more likely to develop CVD and have greater chance of heart disease and stroke. Insulin, a peptide hormone produced by pancreatic β-cells of islets of Langerhans, plays a vital role in regulating the plasma glucose levels. Some previous studies discovered that naringin and hesperidin which belong to the class of flavanones are the major flavonoids found in the *Citrus* fruits. These phytochemicals show pronounced effects in regulating blood glucose levels in diabetic patients via several helpful mechanisms (Alam et al. 2014; Jayaraman et al. 2018).

Hesperetin, an excellent flavanone, was used as an antidiabetic agent in one experiment using STZ-induced rats. From the study, the researchers observed that hesperetin is able to help in metabolic syndrome as it significantly reduces the blood glucose level to the normal value. Hesperetin can improve the structure of β-cells of the islets of Langerhans in the pancreas and definitely improve their survival and therefore make them able to continuously produce and secret insulin, a potent

hypoglycemic hormone that controls to normal basal level of both prandial and postprandial plasma glucose level. This statement is proven during the study; the researchers have conducted an immunohistological examination to precisely investigate the structure of β-cells (Jayaraman et al. 2018).

Moreover, stable structure of pancreatic β-cells will eventually contribute to high synthesis of insulin, and consequently more insulin will be secreted to the bloodstream. Insulin will then bind to the insulin receptor on its target cells such as liver cells and skeletal muscle cells. The binding of insulin to α-subunits of tyrosine-kinase receptor on skeletal muscle cells causes the autophosphorylation of β-subunits and further activates the intracellular signaling pathway. The activation of this pathway helps the mobilization of glucose transporter 4 (GLUT4) to the cell membrane and therefore enhances the entry of glucose into the cells resulting in reduction of plasma glucose level. According to Zygmunt et al. (2010), they suggest that naringenin exhibits its hypoglycemic activity by increasing the uptake of glucose into cells via upregulation of AMP-activated protein kinase (AMPK) signaling pathway in skeletal muscle cell.

Apart from that, hesperetin can also ease the activity of glucokinase enzyme because ultimately hesperetin increases the production of insulin. Glucokinase is an enzyme that facilitates the phosphorylation of glucose to glucose-6-phosphate and mainly involve in glycolysis pathway. Improvement of glucokinase activity eventually deputes the efficient consumption of glucose and enhances the formation of energy through glycolysis pathway resulting in attenuation of glucose concentration in circulation.

Hesperetin extensively increases the production and secretion of insulin into the circulation, and it significantly reduces the level of gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase (Jayaraman et al. 2018). Both of these enzymes play their role in regulating the production of glucose through the gluconeogenesis pathway. Since hesperidin could taper off the level of gluconeogenic enzymes, it absolutely subsides the fabrication of glucose in the body and hence declines the risk of people from developing hyperglycemia. To summarize, both *Citrus* flavonoids naringenin and hesperetin reveal a very promising antihyperglycemic potential that can prevent the development of CVD events (Fig. 22.3).

22.4 Conclusions

In conclusion, *Citrus* is a genus in the family of Rutaceae, and is mainly produced in the regions of the Northern Hemisphere, and its annual global production has been rapidly growing in parallel to the high demand from the consumers. *Citrus* fruits have many phytonutrients and phytochemicals, which can be extracted from its albedo part which contains flavanones, *naringin*, and *hesperidin*. Some of the biological properties have been studied from these fruits, which make them able to act as prevention from cardiovascular diseases (CVDs) including antihypertensive, anti-hypercholesterolemia, and antidiabetic. Antihypertensive effect is regulated by several mechanisms including production of nitric oxide (NO), reducing the activity

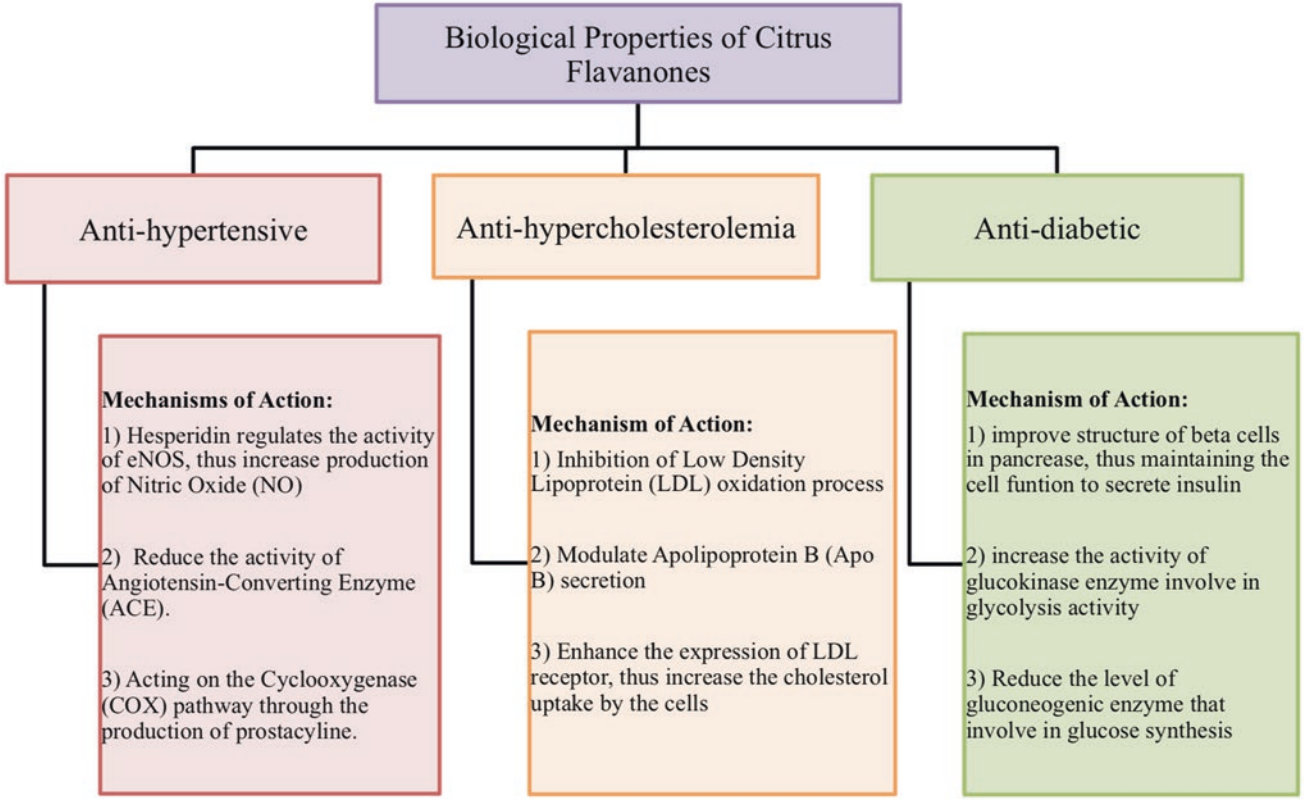


Fig. 22.3 Summary of biological properties of *Citrus* flavonoids

of angiotensin-converting enzyme (ACE) and also acting on the cyclooxygenase (COX) pathway to produce prostacyclin. The consequences of these mechanisms lead to the reduction of blood pressure. Next, for anti-hypercholesterolemia, the flavanones act by inhibiting the low-density lipoprotein (LDL) from oxidation, able to modulate apolipoprotein B (ApoB) secretion, and also by enhancing the expression of LDL receptor and therefore increase the uptake of cholesterol into cells. Lastly, as for antidiabetic, it is found that hesperetin is able to improve the structure of beta-cells in the pancreas, which then increase the cell's integrity to continuously function. Apart from that, hesperetin also helps to speed up the activity of glucokinase enzyme and reduce the level of gluconeogenic enzyme. In a nutshell, it is hoped that further study will be conducted on *Citrus* fruits as to explore more about their potential beneficial effects especially in improving the health status as it is one of the many fruits that can easily be reached by the consumers, due to their strong annual production around the globe.

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Bioflavonoids as Promising Antiosteoporotic Agents

23

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Contents

Abstract

Osteoporosis is a vertebral and nonvertebral skeletal disease, which is characterized by decrease in bone mass, fragile bones, and fractures. Osteoporosis shows a catastrophic effect on the lives of women after menopause. It worsens the life quality with a considerable morbidity and demise. Therefore, its prevention and management are of prime importance. Plant-based products are well documented

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for their antiosteoporosis properties, since ancient times. In the last decade, natural products, including bioflavonoids, terpenoids, lignin, and coumarins have been identified as important resources to develop new therapies for the treatment of osteoporosis. Also, dietary supplements are useful in preventing and control of osteoporosis. Bioflavonoids (or flavonoids) are a diverse and vast group of phytochemicals of over 4000 compounds, ubiquitously distributed in vegetable, medicinal plants, and herbs. Flavonoids are an important constituent of the human diet, and are present in various types of fruits and vegetables, such as onions, berries, kale, grapes, Brussels sprouts, citrus fruit, parsley, and many spices. Bioflavonoids modulate the enzymatic activity and behavior of many cell systems and signaling pathways, suggesting that the compounds may possess a significant antiosteoporotic activity. This chapter summarizes recent reports on the isolation of flavonoids with antiosteoporotic agents from different medicinal plants and foods with a particular emphasis on chemical structures and pharmacological data related to the treatment of osteoporosis.

Keywords
Bioflavonoids · Flavonoids · Osteoporotic agents · Medicinal plants

23.1 Introduction

Osteoporosis is a vertebral and nonvertebral skeletal disease, which is characterized by fragile bones, fractures, and low bone mass by the decrease in the ratio of bone mineral to the organic matrix (Simon 2005). Osteoporosis is also known as a silent disease, because of unnoticeable symptoms in the body. It is difficult for a person to know that he/she is having osteoporotic type of problems until bone braking or fracture, due to unexpected crash or fall. The first symptom of osteoporosis might be felt in the form of loss of tallness and intense back pain. The whole process of growth of the bones (bone modeling) or bone metabolism (bone remodeling) takes place in bone multicellular units (BMUs), which was firstly explained by Frost and Springfield (1963). Osteoporosis is a global problem, and it was estimated to surge from about ten million individuals to over 14 million individuals by the year 2020 in the USA alone (Burge et al. 2007). Although it could be diagnosed in older men, it is predominantly found in women, because of change in hormones and growth factors, mainly after the menopause. Estrogen and testosterone show bone modeling/remodeling effects by inhibiting bone breakdown, and thus control the health of bone in both women and men. The parathyroid hormone (PTH) or parathormone, released by the parathyroid gland in the neck, controls the calcium levels in the body and hence plays vital roles in the formation of bones via improving the multiplication and differentiation of osteoblasts (Tu et al. 2018). Receptor activator of nuclear factor kappa-B ligand (RANKL), also known as nuclear factor kappa-B (NFkB) ligand, is expressed in the osteoblasts, liver, skeletal muscles, small

intestine, and other organs. It binds with RANK receptors on osteoclasts and has been identified to take part in osteoclast activation and maturation, which result in bone resorption. The activated osteoclasts secrete cathepsin K (CatK) in the course of bone resorption process. CatK also plays a role in bone matrix as well as mineral components of bone tissue degradation (Bozec and Zaiss 2017). For a long time, minerals (e.g., calcium and phosphate) and hormone-based therapies have been used clinically as effective medications. One of the best therapies is the estrogen therapy (ET) for treating osteoporosis, where replacement with estrogen induces osteoblast differentiation, promotes bone deposition, and inhibits osteoclasts (Bansal et al. 2013). However, literature survey showed that estrogen and related therapies might cause higher incidences of endometrial cancer and breast cancer, in addition to numerous side effects (Wronsky et al. 1993; Canalis et al. 1998; Constantine et al. 2019). Therefore, the prevention of osteoporosis is more favorable than treatment, which includes regular physical exercise and intake of dietary supplement. Hence, there is an utmost need for developing novel molecules/drugs possessing minimum side effects to prevent osteoporosis. Also, new drugs can become a substitute or lessen the requirement for chemical drugs that are currently in use. Since ancient times, natural products are being used in medications against various ailments. Among the major sources of new chemical entities for drug development include the natural resources, including plants, microbes, lichens, etc. Plants are widely utilized for the treatment of different diseases, including osteoporosis (Sofowora et al. 2013; Arumugam et al. 2016; Swamy et al. 2016, 2017; Mohanty et al. 2017). The medicinal plants and their extracts in the form of decoction, paste, and enriched fractions have been used for osteoporosis evasion and management in the traditional systems of medicines, including Ayurveda, Unani, Siddha, and Chinese since ancient times. Currently, more than 40% of all known drugs are natural products or their derivatives thereof. There are several classes of plant-derived substances. Among them, flavonoid or bioflavonoid is one of the prevalent classes, which is referred as "phytoestrogens," due to having the ability to bind with the estrogen receptor (ER), and thereby decreases bone loss. This chapter deals with the recent developments in the search and development of flavonoids as potent antiosteoporotic agents.

23.2 Classification of Osteoporosis

Based on diagnosis, etiology, or stage of disease, osteoporosis can be classified in two classes: primary osteoporosis and secondary osteoporosis. The primary osteoporosis is of two types, i.e., type I osteoporosis and type II osteoporosis. The classification is done on the basis of actual levels of calcium mineral left in the skeleton. In menopausal women's body, the levels of estrogen are critically diminished, and hence they are more prone to develop type I osteoporosis, which is also usually identified as postmenopausal osteoporosis. In this process, bones lose the matrix or substance due to a surge in the resorption of bone. Approximately, 5–20% of women, most often between the ages of 50 and 75, suffer type I osteoporosis,

because of low estrogen level after menopause. As a result, quick reduction of calcium from the skeleton takes place. Type I osteoporosis is also associated with cracks (fractures) that arise in the spine, vertebrae, hip, wrist, forearm, etc. Type II osteoporosis (senile osteoporosis) generally arises on or after 70 years of age. This type of osteoporosis commonly affects women in comparison to men. In this, bone breakdown overcomes the bone formation after a disturbance in the process of resorption as well as formation of bone, frequently causing various bone fractures, including vertebrae, neck, humerus, and pelvis fractures. As a consequence, either declined vitamin D synthesis or vitamin D resistance takes place. This is most likely intermediated by declined or insensitive vitamin D receptors in selected osteoporotic patients. The incidence of secondary osteoporosis is very low and found in approximately less than 5% cases. Secondary osteoporosis arises, due to endocrine diseases, including hyperthyroidism, hypogonadism, hyperprolactinemia, diabetes mellitus, etc. It may also be originated either from overdoses of some drugs like glucocorticoids, Dilantin, barbiturates, tobacco, and heparin or from other multifarious circumstances like liver disease, chronic renal failure, malabsorption, sarcoidosis, chronic obstructive in lungs, malignancy, as well as persistent weightlessness as observed in the spaceflight.

23.3 Flavonoids: Occurrence and Classification

Flavonoids, including over 6000 identified members, establish an important class of biologically active constituents from plant resources (Yu et al. 2016). Flavonoids are universally distributed in the plant kingdom, including fruits, vegetables, and herbs. Most commonly, they are found in parsley, thyme, onions, cherries, apples, broccoli, kale, buckwheat, tea, green tea, grapes, berries, wine, oranges, grapefruit, soya beans, and legumes. Flavonoids possess a common basic skeleton having phenyl benzopyrone structure of C6-C3-C6 carbon atoms. They are classified on the basis of the carbons of the C ring on which the B ring is attached, oxidation pattern of the C ring, and degree of unsaturation, mainly into flavones, flavonols, flavans, flavanones, flavanonols, and isoflavones as shown in Fig. 23.1. Isoflavonoids are flavonoids, when ring B is directly bound to the position 3 of the ring C. On the other hand, when ring B is directly linked to position 4 of the ring C, they are called as neoflavonoids. Flavones and flavonols are usually found in plants as *O*-glycosides or *C*-glycosides, whereas glycosides of flavanols are rare in nature (Williams and Harborne 1994). Free flavonoids, as the aglycone, are not attached to any sugar residue. They are not present in fresh plants but may be originated as a result of food processing reactions in processed foods. In most of the cases, sugars are predominantly linked to the flavonoid nucleus via a β-glycosidic bond at various positions preferably C3 position of the parent skeleton. More than 80 different sugars, such as monosaccharides, disaccharides, trisaccharides, and even tetrasaccharides are found in the form flavonoid glycosides in the plant kingdom (Finger et al. 1991).

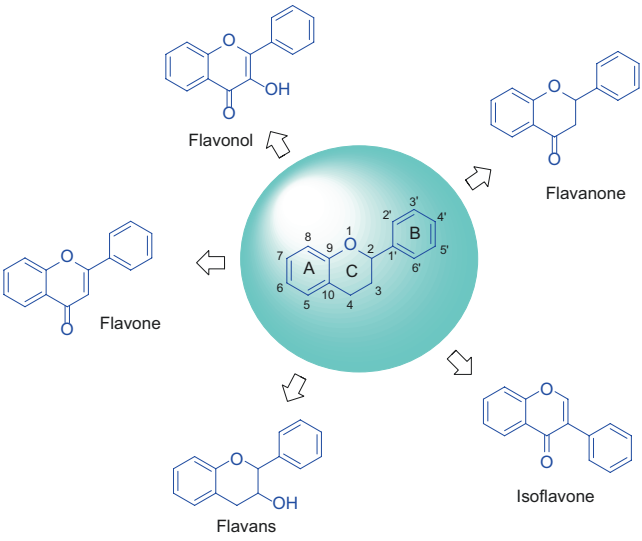

Fig. 23.1 Classification and basic structures of flavonoids

Flavonoids have been shown as protective mechanisms in various biochemical pathways, which are linked with many diseases like Alzheimer's disease (AD), Parkinson disease (PD), cancers, atherosclerosis, cardiovascular, neuroprotection, oxidative stress, etc. This is mainly because of their antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, and many other pharmacological properties. Flavonoids also can function as a potent inhibitor or activator of a number of enzymes, including cyclooxygenase (COX), xanthine oxidase (XO), lipoxygenase, diamine oxidase (DAO), phosphoinositide 3-kinase, and monoamine oxidase (MAO), involved in several biochemical reactions. Thus, as health promoters, flavonoids are extensively used as pharmaceutical and nutraceutical agents for medicinal and cosmetic uses in the form of variable and indispensable components.

23.3.1 Flavones

Flavones are the largest class of flavonoids with a double bond between C2 and C3 in the basic carbon skeleton. At the C3 position, they do not have substitution but show the presence of ketone group at C4 position. They play numerous roles in plants including providing pigmentations to the flowers and other parts in the form of anthocyanins, protection from ultraviolet light, acting as natural pesticides, promotion of colony formation of nitrogen-fixing bacteria, and also serving as signaling molecules in biochemical pathways. High concentration of flavones is found in leaves, flowers, and fruits as glycosides. As shown in Fig. 23.2, major sources of flavones are chamomile flowers (5320 mg as apigenin O-glycosides/100 g dried weight), dried parsley leaves (1350 mg as apigenin O-glycosides/100 g dry weight),

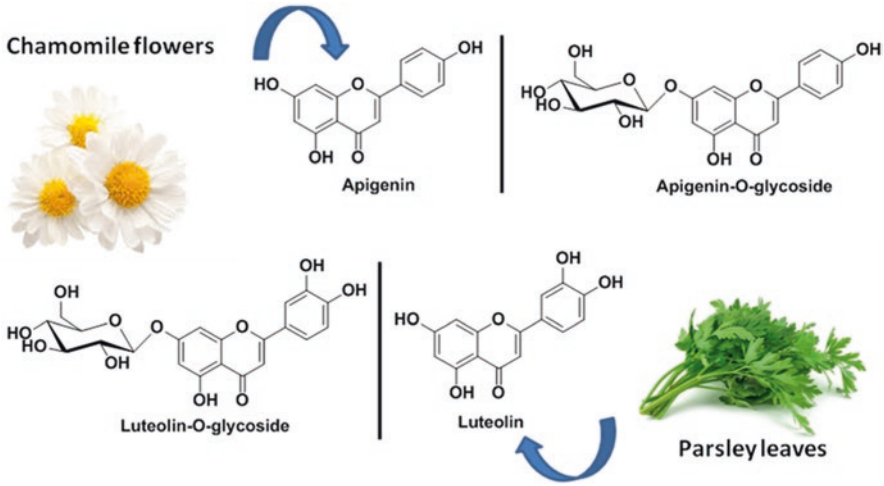

Fig. 23.2 Some important examples and sources of flavones

fenugreek seed (731 mg as apigenin and 512 mg as luteolin per 100 g dry weight), peppermint leaves (42–3070 mg as luteolin glycosides per 100 g dry weight), oregano (901–1137 mg as luteolin per 100 g dry weight), rosemary (10–661 mg as luteolin O-glycosides per 100 g dry weight), and green tea (47.8–246.8 mg as apigenin C-glycosides per 100gm dry weight) (Gregory et al. 2017). Small amount of different flavones are also found in oranges, citron juices, grapefruit peels, spinach, celery, lettuce, broccoli, berries, olive oil, etc. Fresh parsley had the highest concentrations of apigenin (≤1484 mg/100 g) (Mattila et al. 2000), whereas chicory had luteolin in significant amount (≤333 mg/100 g) (Arabbi et al. 2004).

23.3.2 Flavonols

Flavonols show similar structure as flavones, having a double bond at the C2–C3 position, but unlike flavones, they have −OH group at C3 position. These compounds are the building blocks of proanthocyanidins. Major naturally occurring flavonols include quercetin, kaempferol, myricetin, fisetin, etc., which are observed in a range of edible vegetables and fruits (Fig. 23.3). High concentration of quercetin is found in the fresh weight of onions (2.80–4.90 mg/g), kale (1.10 mg/g), broccoli (0.3 mg/g), broad beans (0.45–0.60 mg/g), apples (0.20–0.70 mg/g), apricot (0.25 mg/g), and blackcurrants (0.37 mg/g) (Hertog et al. 1992). Moreover, kaempferol is found in kale (2.10–4.70 mg/g), broccoli (0.60 mg/g), black tea infusion (0.14–0.16 mg/g), and strawberry (0.05–0.12 mg/g) in significant amount. The content of myricetin is generally low including in broad beans (0.30 mg/g), black grapes (0.045 mg/g), black tea infusion (0.03 mg/g), and red wine (0.079 mg/g) (Crozier et al. 1997). Flavonols have been found to exhibit wide-ranging health-promoting

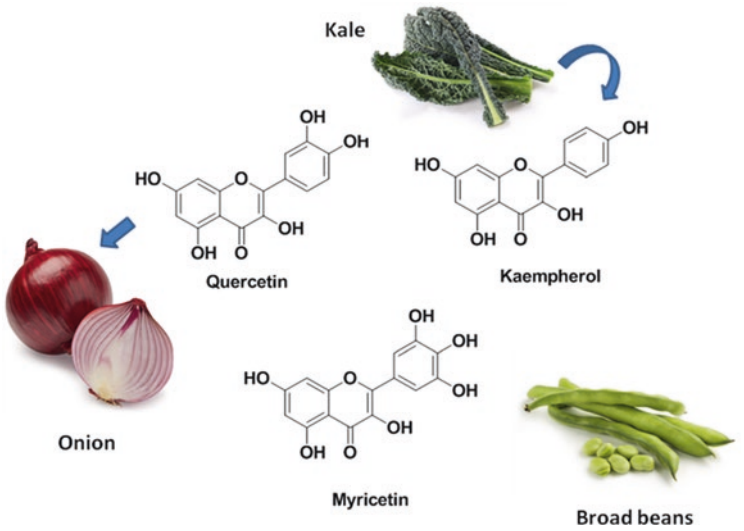

Fig. 23.3 Some important examples and sources of flavonols

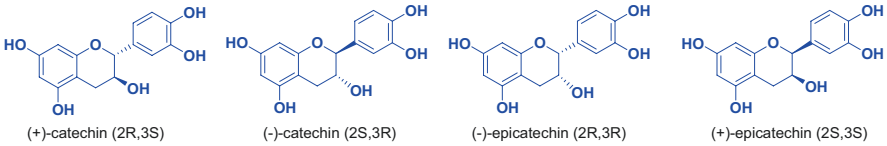

Fig. 23.4 Structures of four diastereoisomers of catechins

effects, mainly mediated via reducing oxidative stresses. They are found be more effective in the treatment of cardiovascular and neurodegenerative diseases.

23.3.3 Flavanols

Flavanols also have the same basic skeleton as in flavones and flavonols except absence of oxygen at the C4 position of the heterocyclic C ring and double bond at the C2-C3 position. The presence of a hydroxy group at C3 position creates two asymmetric centers (chiral centers) on carbon 2 and 3 and, thus, finally forms four diastereoisomers. Among them, two of the isomers occur in *cis*-configuration, and they are known as epicatechin, while the other two of the isomers exist in *trans*-configuration and are called as catechin (Fig. 23.4). (+)-Catechin is the most frequently occurring catechin isomer. (−)-Catechin (epicatechin) is the other common stereoisomer occurring in nature. So far only *2R* configurations have been observed in the natural sources.

The predominating flavanols are (−)-epicatechin (2R,3R-3,5,7,3′,4′-pentahydroxyflavan) (EC), (+)-catechin (2R,3S-3,5,7,3′,4′-pentahydroxyflavan), (−)-epigallocatechin (EGC) (2R,3R-3,5,7,3′,4′,5′-hexahydroxyflavan), (+)-gallocatechin (GC) (2R,3S-3,5,7,3′,4′,5′-hexahydroxyflavan), and gallic acid esters, such as (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG) (Peter and Hollman 2000). These are found abundantly in fruits, foodstuffs, and beverages, for example, apples, grapes, red wine, tea, and cocoa (Fig. 23.5). Several studies have been found to show that flavanols from cocoa and tea help in improving blood vessel health and facilitate proper circulation of blood for the delivery of oxygen and important nutrients to the various organs and tissues in the body. Green tea is one of the major source of epicatechins as EGCG (~60%), EGC (~20%), ECG (~14%), and EC (~6%), and these compounds are most useful in the treatment of anxiety, depression, influenza, obesity, AD, and PD by hindering amyloid-β plaque development in the brain, along with several forms of cancers (Wanda 2018). It is assumed that one bag of green tea contains approximately 25–30 mg EGCG (Botten et al. 2015)

23.3.4 Flavanones

Flavanones constitute an important class of flavonoids with both aglycone and glycosylated compounds. Their basic skeleton consists of flavan nucleus of two aromatic rings A and B, which are linked by a dihydropyrone ring (C). They have a chiral center at C2 position and oxygen atom at C4 position (Khan et al. 2014). The examples of predominant flavanones are naringenin, hesperetin, isosakuranetin, eriodictyol, and taxifolin in aglycone form, though citrus fruits have glycosylated

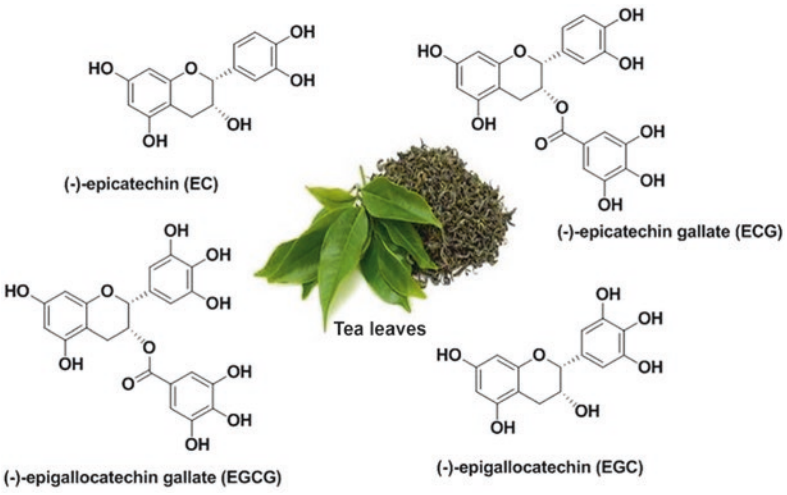

Fig. 23.5 Some important flavanols and their source

derivatives in major amount. For human consumption, they are commonly obtained from juices of hand-squeezed or industrially processed citrus fruits, such as lemons, grapefruits, oranges, mandarins, kumquats, tangors, bergamots, tangerines, etc. (Fig. 23.6). Among the flavanones, hesperidin is the most abundant being present in high concentrations in blood sweet oranges (200–600 mg/L), mandarins (8.1–460 mg/L), *Citrus clementina* (50–850 mg/L), lemon juice (38–410 mg/L), grapefruit (20–170 mg/L), and tangelos (10–70 mg/L). Concentration of hesperetin is present in different juices including lemon (145 mg/L), orange (120 mg/L), and grapefruits (23 mg/L), whereas naringenin is present in different juices in different concentration, i.e., juices of pummelo (253 mg/L), grapefruit (182 mg/L), lemon (182 mg/L), and orange (21 mg/L), respectively (Peterson et al. 2006). Flavanones increase the body's defenses via scavenging reactive oxygen species (ROS) and help in the prevention of cardiovascular diseases, atherosclerosis, and cancer. In addition, they show antimicrobial, anti-inflammatory, antidiabetic, and antiosteoporosis pharmacological activities (Barreca et al. 2017).

23.3.5 Isoflavones

In isoflavones, ring C is attached with the C3 position of ring B. They have a double bond between C2 and C3 position and are substituted with the oxygen atom at the C4 position (Miadoková 2009). They are mainly found in members of the Fabaceae family like *Glycine max* L. (soybean), green *Phaseolus vulgaris* L. (bean), *Medicago sativa* L.(alfalfa), *Vigna radiata* L.(mung bean), and *Vigna unguiculata* L. (white

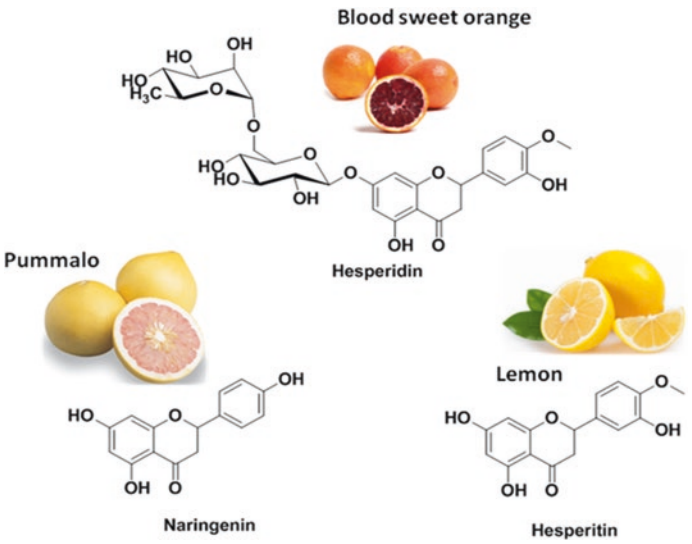

Fig. 23.6 Some important examples and sources of flavanones

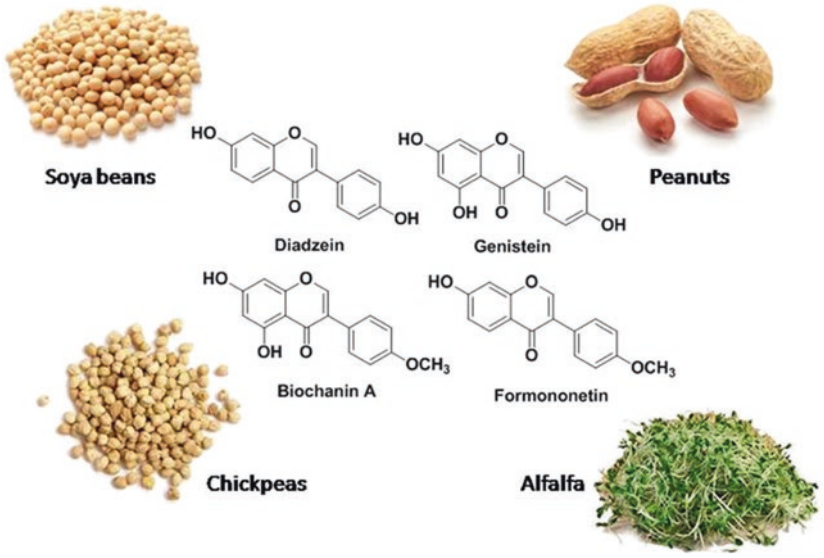

Fig. 23.7 Some important examples and sources of isoflavone

cowpea) and roots of *Pueraria lobata* L., etc. (Fig. 23.7). Isoflavones are the natural phytoestrogens, and they act through pseudohormonal activity by binding to estrogen receptors (ER) in mammals (Messina et al. 2006). They block the binding of estrogens with the receptor to prevent hormone-related diseases like breast cancer and cervical cancer in women whereas prostate or testicular cancers in men (Birt et al. 2001). Moreover, they also have anticancer, antimicrobial, anti-inflammatory, and antioxidative activities. Daidzein, genistein, biochanin A, and formononetin are the most common isoflavones and are obtained from soya bean, peanut, chick pea, and alfalfa, respectively (Hwang et al. 2009).

23.4 Flavonoids as Antiosteoporotic Agents

Several flavonoids from different plants have exhibited antiosteoporotic activity. Prouillet and co-workers determined estrogenic activity of quercetin (**1**) and kaempferol (**2**) through alkaline phosphatase (ALP) marker in human MG-63 osteoblast cell lines. As depicted in Fig. 23.8, compounds **1** and **2** increased the ALP activity via downstreaming of estrogen receptor (ER)-induced stimulation of extracellular regulated kinase (ERK) pathway (Prouillet et al. 2004). Rutin (**3**) was evaluated for its protective effect against osteopenia in ovariectomized (OVX) rats with dose of 2.5 g/kg body weight as dry feed daily for 90 days. On completion of 90 days, OVX rats showed increased femoral strength along with inhibition of estrogen deficiency-prompted femoral trabecular bone loss by decelerating resorption and through enhancing osteoblastic activities (Horcajada-Molteni et al. 2000). *Ulmus*

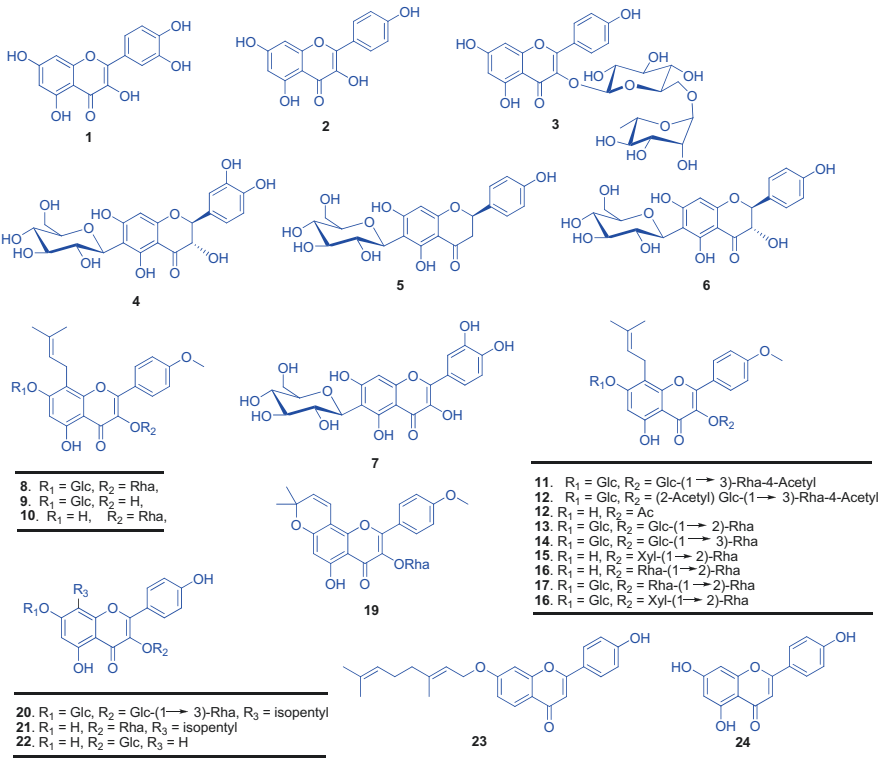

Fig. 23.8 Structures of antiosteoporotic flavonoids isolated from *Ulmus wallichiana*, Er-Xian Decoction, *Epimedium koreanum* Nakai, *Millettia griffoniana*, and others

wallichiana, a bone-healing plant in the Himalayan region of India, is a plentiful source of various types of flavonoids. Four C-glycoflavonoids including (2S,3S)-aromadendrin-6-C-β-D-glucopyranoside (**4**), naringenin-6-C-β-D-glucopyranoside (**5**), (2S,3S)-(+)-40,5,7-trihydroxydihydroflavonol-6-C-β-D-glucopyranoside (**6**), and quercetin-6-C-β-D-glucopyranoside (**7**) were sequestered from *U. wallichiana* stem bark ethanol extract. Osteogenic effect of these compounds has been assessed by alkaline phosphatase marker (Rawat et al. 2009). (2S,3S)-Aromadendrin-6-C-β-D-glucopyranoside increased the gene expression of osteogenic markers like BMP-2, Runx-2, type I collagen, and osteocalcin in the pre-osteoblasts along with the inhibition of osteoclast differentiation at the concentration range of 1 nM to 100 nM in a concentration dependent way (Swarnkar et al. 2011) (Fig. 23.8). This compound thus has been identified as a more potent osteogenic, anti-osteoclastogenic, and anti-adipogenic agent than kaempferol, which was active at μM concentration (Trivedi et al. 2008). Compound **4** exhibited significant osteogenic effects in vitro and bone marrow bioavailability studies as compared to quercetin treatments at osteogenic doses (Siddiqui et al. 2011).

Some important compounds like icariin (**8**), icariside I (**9**), and baohuoside I (**10**) were obtained from the aqueous extract of the Chinese folk medicine formula, i.e., Er-Xian Decoction (EXD). All these compounds **8, 9,** and **10** augmented osteoblast propagation and ALP activity. Further, they also reduced the TRAP activity of osteoclast. In an ovariectomized (OVX) rat model, icariin (**8**) compound improved the bone mineral density, reestablished the histological morphology of bone, and demonstrated a strong antiosteoporotic activity. The compound **8** was found to escalate the formation of bone and inhibition of bone resorption along with significant promotion of differentiation of primary osteoblasts (Qin et al. 2008) (Fig. 23.8).

Fifteen flavonol glycosides, namely, **8**, **10**, korepimedoside C (**11**), caohuoside E (**12**), sagittatoside A (**13**), hexandraside D (**14**), hexandraside F (**15**), sagittatoside B (**16**), 3,5,7-trihydroxy-40-methoxy-8-prenylflavone-3-O-a-L-rhamnopyranosyl-(1–2)-a-L-rhamnopyranoside (**17**), epimedin C (**18**) and epimedin B (**19**), acuminatin (**20**), epimedoside A (**21**), baohuoside II (**22**), and astragalin (**23**) were obtained in pure forms from *Epimedium koreanum* (Berberidaceae) aerial parts and characterized for their chemical structures (Zhang et al. 2008a, b). This herb is very commonly utilized in Chinese medicines for treating impotence and osteoporosis problems. Compounds **8** and **10–23** exhibited a strong inhibition on the multiplication and differentiation of rat calvarial osteoblast. These results were evaluated by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) method via measurement of ALP activity (Zhang et al. 2008a, b) (Fig. 23.8). Griffonianone E (**23**) isolated from *Millettia griffoniana* (Leguminosae) roots and stem bark was evaluated for its activity in a yeast-based estrogen receptor alpha (ERα) assay, and it was found as a significant activator of β-galactosidase enzyme (Ketcha et al. 2006). ALP activity of **23** was also found at the concentration of 10^{-5} M in human cultured cells, such as Ishikawa and MVLN cells. In another study, apigenin (**24)** inhibited TNF-α and IFNγ-persuaded discharge of many osteoclastogenic cytokines from MC3T3-E1 (mouse osteoblastic cells). In addition, **24** intensely repressed the differentiation of 3 T3-L1 preadipocytes to adipocytes with attendant suppression of adipocyte differentiation-induced production of MCP-1, IL-6, and leptin. The presence of **24** also exhibited the inhibition of osteoclast differentiation from the RAW 264.7 cells by downregulating RANKL-induced expression of tartrate-resistant acid phosphatase (TRAP), RANK, and calcitonin receptors. It resulted into the suppression of multinucleated osteoclast formation. In a similar study, **24** inhibited expressions of the differentiation markers of osteoclast, such as RANK, TRAP, and c-Fms in osteoclast precursor cells that were obtained from the bone marrow of mouse treated with macrophage colony-stimulating factor (MCSF) and RANKL. Furthermore, **24** was also found to stimulate the apoptosis of mature osteoclasts of long bones of rabbit along with the inhibition of bone resorption. Thus, apigenin had manifold special effects on all the bone cells in vivo, suggesting its possible use in preventing bone loss (Bandyopadhyay et al. 2006) (Fig. 23.8).

Cephalotaxus koreana Nakai aerial parts were extracted with a solvent, ethanol, to obtain several compounds (**25**–**32**), which were characterized for their chemical structures. Among them, isoscutellarein 5-*O*-*β*-D-glucopyranoside (**28**), kaempferol 3-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*β*-D-glucopyranoside (**29**), tamarixetin

3-*O*-α-L-rhamnopyranosyl-(1‴ → 6″)-*β*-D-glucopyranoside (**30**), quercetin 3-*O*-(6″-*O*-acetyl)-*β*-D-glucopyranoside (**31**), and quercetin 3-*O*-α-L-rhamnopyranoside (**32**) exhibited substantial inhibitory property against osteoclast differentiation at concentrations ranging between 0.1 and 1.0 μg/mL. As a result, TRAP-positive multinucleated cell inhibition was found to be more than 50% (Yoon et al. 2007). Problems related to sex hormones and aging process may lead to inflammations and oxidative conditions that might be involved in osteoporosis pathogenesis. Recent investigations have reported that polyphenols might exercise shielding effects in such situations. Phloridzin (**33**), a flavonoid exclusively occurring in *Malus domestica* (Rosaceae), was shown to prevent ovariectomy-prompted bone loss in situations of inflammations as revealed by bone mineral density in OVX rats. At the diaphyseal site, bone mineral density was found to improve significantly with or without inflammations. Also, it is suggested that a daily intake of phloridzin may provide shielding effect against ovariectomy-induced osteopenia under inflammation circumstances through the improvement of inflammatory markers and bone resorption (Puel et al. 2005) (Fig. 23.9).

Amentoflavones (a type of bioflavonoids), namely, bilobetin (**34**), ginkgetin (**35**), 4′,7″-di-*O*-methyl-amentoflavone (**36**), 7-*O*-methyl-isoginkgetin (**37**), sciadopitysin (**38**), and 7,4′,7″,4‴-*O*-methyl-amentoflavone (**39**), were isolated and identified from ethyl acetate fraction of *Cephalotaxus koreana* (Cephalotaxaceae) leaves and twigs. Compounds **34**, **37**, **38**, and **39** exhibited significant improvement of ALP activity at doses ranging between 1.0 and 20.0 μM. However, **35** and **36** showed lower ALP activity at 20.0 μM dosage. The effect of **34**, **37**, **38**, and **39** on ALP activity was also envisaged by ALP staining. Moreover **34** and **39** significantly increased the synthesis of collagen, whereas **38** exhibited higher level of synthesis of collagen up to 180% compared to control cells at 10.0 μM concentration. In

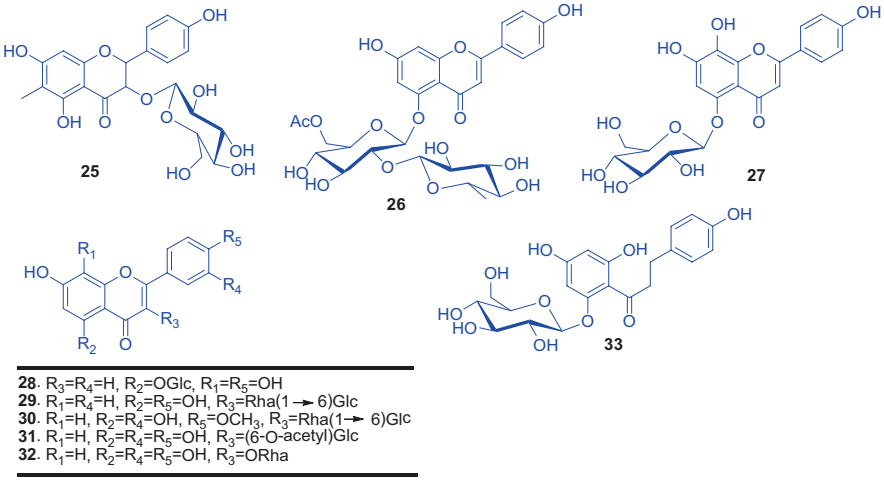

Fig. 23.9 Structures of antiosteoporotic flavonoids isolated from *Cephalotaxus koreana* Nakai and *Malus domestica*

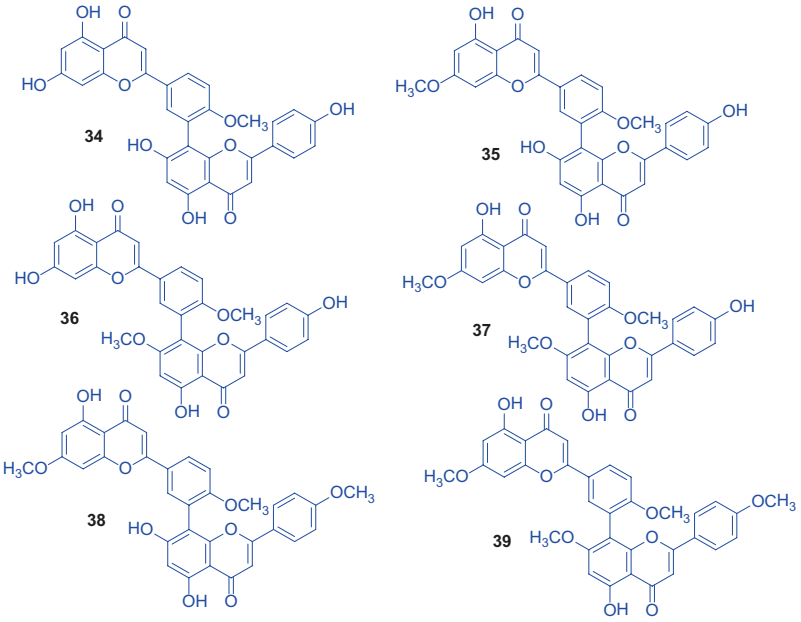

Fig. 23.10 Structures of antiosteoporotic biflavonoids isolated from *Cephalotaxus koreana* Nakai

another experiment, compounds **34**, **38**, and **39** were found to show significant stimulatory effect on mineralization as detected using Alizarin red staining method (Lee et al. 2006). Cathepsin K, a protease enzyme, has high specificity for kinins that are involved in bone resorption. Cathepsin K catabolizes the elastin, collagen, and gelatin fibers and thus takes parts in breaking of bone and cartilage. Therefore, flavonoids having inhibition of cathepsin K enzyme show great potential in the treatment of osteoporosis (Fig. 23.10).

Biflavone amentoflavone **(40)**, 4‴-methyl amentoflavone **(41)**, 7″,4‴-dimethyl amentoflavone **(42)**, 4′-methyl amentoflavone **(43)**, 2,3-dihydroamentoflavone **(44)**, and hinokiflavone **(45)** isolated from *Taxodium mucronatum*, *Cycas guizhouensis*, *Murraya koenigii*, and *Winchia calophylla* are natural inhibitors against lysosomal proteases "cathepsin K" with IC_{50} below 10 μg/ml (Zeng et al. 2006). Six biflavones **34** and **40–44** isolated from different plants potently inhibit cathepsin B in vitro. Especially compound **42** and **43** can inhibit cathepsin B with IC_{50} of 0.62 and 0.58 μM, respectively (Zenga et al. 2006) (Fig. 23.11). 8,8″-Biapigenin (**46**), a condensation product of two apigenin molecules, was isolated and characterized as a major biflavonoid from the nuts of *Cupressus sempervirens*. **46** was observed to encourage osteoblast proliferation, differentiation, and mineralization mediated via estrogen receptors (ER) as antiestrogen at 10^{-10} M concentration on murine bone cells in vitro and also in vivo using OVX mice. It also inhibited the osteoclastogenesis and differentiation of 3 T3-L1 of bone marrow cells at 10^{-10} M and 10^{-8} M. It was reported to inhibit the mRNA levels of osteoclast genes, such as RANK, TRAP,

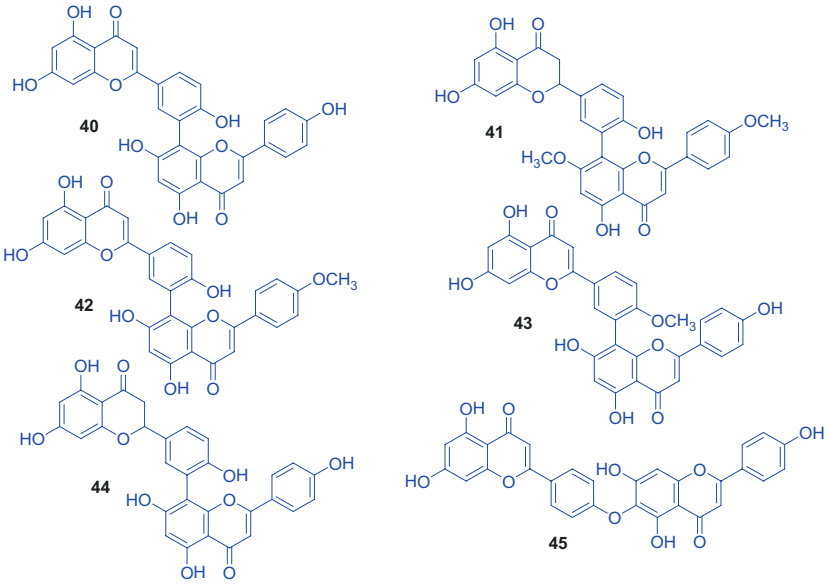

Fig. 23.11 Structures of antiosteoporotic biflavonoids isolated from *Taxodium mucronatum*, *Cycas guizhouensis*, *Murraya koenigii*, and *Winchia calophylla*

IL-6, and TNF-α, and RANK ligand/osteoprotegerin ratio in bones compared with OVX mice treated with vehicle. It also induced the formation of osteoprogenitor cells in the bone marrow and enhanced the levels of mRNAs of osteogenic gene core binding factor α-1, type I collagen, and BMP-2 in bones in comparison to OVX + vehicle group (Siddiqui et al. 2010). A study published by Maurya et al. research group evaluated that isoflavonoids such as cajanin (**47**), formononetin (**48**), isoformononetin (**49**), and cladrin (**50**) showed increasing proliferation, differentiation, and mineralization of osteoblast cells via increased expression of alkaline phosphatase (Maurya et al. 2009). In another study, Bhargavan et al. showed that compounds **47** and **49** isolated from stem bark of *Butea monosperma* exhibited osteogenic activity. Both compounds had strong mitogenic as well as differentiation-promoting effects on osteoblast cells through activation of MEK-ERK and Akt pathways when female SD rats were treated with each of these compounds at 10 mg/kg/day for 30 consecutive days. Additionally, **47** also increased bone mineral densities (BMD), bone biomechanical strength, mineral apposition rate (MAR), and bone formation rate (BFR). Compound **49** was found to be less active (Bhargavan et al. 2009). Moreover, Chattopadhyay et al. have also shown the skeletal effect of **47** obtained from stem bark of *Butea monosperma* on osteopenic rats at 10 mg/kg dose for 4 weeks, after withdrawal. In these results, **47** has been found to show improvement in trabecular microarchitecture, lumbar vertebral strength, bone formation rate (BFR), and cortical thickness. **47** was found to enhance the expression of osteogenic genes and reduce expression of bone osteoclastogenic genes in bones (Khan et al. 2013) (Fig. 23.12).

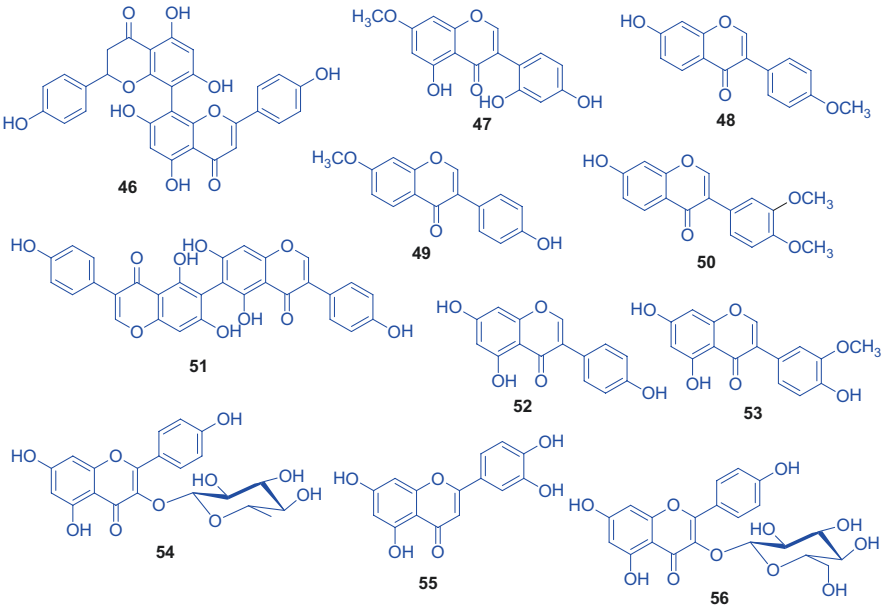

Fig. 23.12 Structures of antiosteoporotic biflavonoids isolated from *Cupressus sempervirens*, *Butea monosperma*, *Podocarpium podocarpum*, and *Winchia calophylla*

Ma et al. (2013) isolated one bi-isoflavonoid, two isoflavonoids, and three flavonoid compounds namely, podocarnone (**51**), genistein (**52**), pratensein (**53**), afzelin (**54**), luteolin (**55**), and astragalin (**56**) from *Podocarpium podocarpum* (Fig. 23.12). Compound **51** stimulated ALP activity, osteoblastic multiplication, and inhibitory effects on osteoclastic TRAP activity at a dose from 10^{-7} to 10^{-9} mol/l. The activity of **51** was equivalent to **52,** which is an established phytoestrogenic compound. In this study, **51** increased the osteoblastic ALP activity up to 15.46% at higher concentration (10^{-12} mol/l) while **52** enhanced same activity up to 17.08% at much lower concentration i.e., 10^{-7} mol/l. Other compounds, i.e., **54**, **55,** and **56** showed a moderate ALP activity in the range between 10^{-7} and 10^{-9} mol/l concentration. However, **53** had no effect on osteoblastic ALP activity at any concentration. All the isolated compounds were found to show significant suppression of the TRAP activity with inhibitory rate of nearly 10% at 10^{-7} mol/L concentration, except for compound **53**. Thus these results showed that compound **51** demonstrated stronger effects both on osteoclasts and osteoblasts at relatively lower concentrations (Ma et al. 2013). Dixit and co-workers isolated 14 isoflavones and flavanols from the ethanolic extract of *Dalbergia sissoo* leaves and identified them as **52**, **53**, biochanin A (**57**), biochanin A 7-O-glucoside (**58**), biochanin A 7-O-(*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside) (**59**), biochanin A 7-O-(*β*-Dapiofuranosyl-(1 → 5)-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside) (**60**), genistein 8-C-*β*-D-glucopyranoside (**61**), caviunin (**62**), caviunin 7-O-*β*-D-glucopyranoside (**63**), caviunin 7-O-(*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside) (**64**), kaempferol 3-O-*β*-D-glucopyranoside (**65**), kaempferol

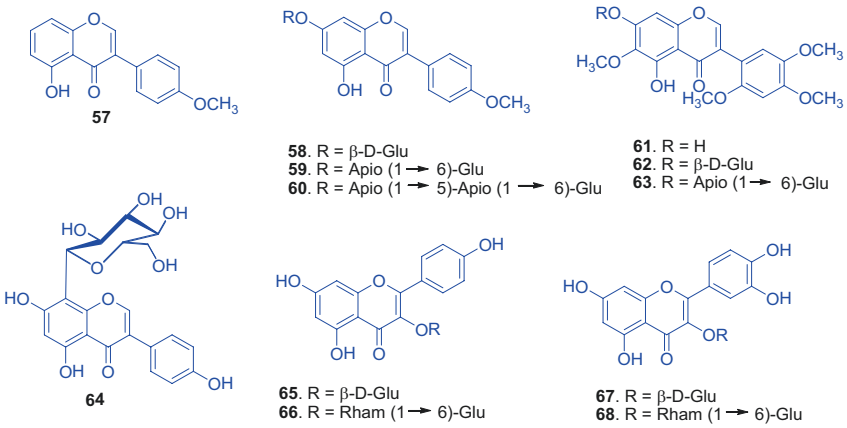

Fig. 23.13 Structures of antiosteoporotic biflavonoids isolated from ethanolic extract of *Dalbergia sissoo* leaves

3-O-rutinoside (**66**), quercetin 3-O-*β*-D-glucopyranoside (**67**), and quercetin 3-O-rutinoside (**68**). All compounds were evaluated for osteogenic activity. Among them compounds **52**, **53**, **57,** and **64** exhibited osteoblast differentiation as assessed by increased ALP and mineralization in cultured primary calvarial osteoblast cells. Compound **52** and **64** also significantly increased the expression of osteogenic gene BMP-2 (Dixit et al. 2012) (Fig. 23.13).

23.5 Conclusions, Present and Future Prospects

Because of growing concern for bone health and high cost and limitations of present therapy, researchers worldwide are making intense efforts to design and develop cost-effective therapeutics and therapies for the treatment of osteoporosis. In this regard, phytochemicals are expected to have a major role in the coming years. Among phytochemicals, flavonoids and their derivatives are especially important in this regard. Many flavonoids possess osteogenic properties like binding with estrogen receptor (ER) and differentiation and mineralization of osteoblast and osteoclast cells through upregulation and downregulation of MEK-ERK and Akt pathways, Runx-2, BMP-2, type I collagen, RANKL, M-CSF, TRAP, TNFα, IFNγ-induced secretion, MCSF, as well as cathepsin K inhibition. Many constituents are in the process of preclinical evaluation or in the consequent phases of clinical trials or in the market as a formulation of plant extract. Based on extensive literature survey, some flavonoids or plant extracts having flavonoids are known to serve as antiosteoporotic agents. For example, caviunin 7-*O*-(*β*-D-apiofuranosyl-(1–6)-*β*-D-glucopyranoside identified as a novel osteogenic marker in the standardized extract of *Dalbergia sissoo* has been launched in the market as “reunion” for rapid

fracture healing and as a therapeutic for primary osteoporosis by Pharmanza Pvt. Ltd., India. The extract of *Ulmus wallichiana* has been developed and licensed by CSIR-CDRI, Lucknow, India along with M/s Kemxtree LLC (USA) company in 2012 for further development as a rapid fracture-healing oral drug. From the discussion in this chapter, it is evident that flavonoid-based scaffolds could serve as potential scaffolds for development of effective novel therapeutics for fracture healing and improvement of bone health. The abundant sources of flavonoids including terrestrial plants, various foods, and vegetables give opportunities for discovery and development of novel flavonoid-based entities with antiosteoporotic activity. We also need to improve the techniques for isolation and purification so as to obtain the naturally occurring flavonoids in higher yield. Moreover, further studies are needed to further evaluate and investigate the possible applications of flavonoids and their mode of action against osteoporosis.

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Computational Approaches in Drug Development and Phytocompound Analysis

24

Glaucia C. Pereira

Contents

Abstract

A plethora of therapeutic properties can be attributed to phytocompounds, and increasing new methodologies can be employed to optimise their utilisation. In cancer research, variations of molecular docking have been employed for mapping protein-phytochemical interactions, seeking alternative approaches to chemotherapy, radiotherapy, and surgery. In neurodegenerative pathologies, progressing research on phytotherapies may lead to effective substitutes for palliative treatments. In Parkinson's disease, for instance, temporary relief results from usage of dopamine agonists, mimicking wild-field dopamine action, in the brain, and stimulating dopamine receptors. On the contrary, plant secondary metabolites are promising, offering long-term modulation of biomarkers for neuro-dysfunction. For nearly a decade, the World Health Organization (WHO) has been reporting on critical trends in cancer, cardiovascular and neurological disorders. According to WHO, in 2005, neuropathologies resulted in 92 million DALYs (disability-adjusted life years), a measure of disease burden, in terms of reducing life expectancy. The figures are projected to peak at 103 million in

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2030, representing 12% increase. The predictions for Alzheimer and other dementias indicate 66% increase, between 2005 and 2030. Therefore, it urges finding effective measures to mitigate these trends. In 2017, regenerative medicine saw 5H-purin-6-amine from *Sedum sarmentosum* extracts, modulating signalling pathways, contributing to increase of spermatogonial stem cell (SSC) activity. This is key in fertility studies, because 5H-purin-6-amine seems to induce SSC concentration within germ cells. However, the benefits of increasing phytochemical extracts-driven stem cell activity go beyond that, with the high-mobility group box 1 (HMGB1) acting as a key upstream mediator in tissue regeneration. Computing is fast becoming a key instrument in drug discovery. Big data analytics and process automation are the domain knowledge revolutionising the field. On one hand, governing large volumes of heterogeneous information challenges drug development. On the other, it brings about rich sources of insights employed in unveiling new medicinal phenotypes and repurposing existing ones. One singular example derives from ontologies used to characterise molecular compounds, forming the basis of large annotated standardised information systems where medicinal compounds are assembled, in dictionaries (e.g. SNOMED) and semantic networks (e.g. Unified Medical Language System). Undoubtedly, the ability to correlate this data is fundamental, endowing conventional systems with cognitive power (e.g. biotechnology empowered by cognitive analytics), to analyse genotype-phenotype pairs, target compounds' interactions, and track diseases' biomarkers, to both elucidate and designate potential ligand-receptor binding prone to modulate dysfunction in diseases' signalling cascades. Within this ambit, this chapter discusses how technology has revolutionised pharmacology. Special attention is given to phytocompound interactions and their role in drug development.

Keywords

Drug discovery and development · Computing · Artificial intelligence · Quantum computing and intractability · Deep learning · Reinforcement learning · Omics · Phytocompound analysis · Molecular docking · Medicinal dictionaries and semantic networks · Data mining · Phenotype-genotype analysis · Evolutionary algorithms · Generative adversarial networks

24.1 Introduction

A plethora of computational frameworks have been designed to accelerate drug development and discovery, at scale. The choice of methodology and infrastructure is driven by specific stages in drug development—e.g. Stage I trials and Hit to Lead (H2L); compounds characteristics—e.g. small molecules phenotypes and immunotherapeutics signalling cascades in autoimmunity; and both data type and volume of information available.Significant progress is observed in drug development, with

myriad of computational approaches empowering existing methodologies—e.g. statistics and bioinformatics founding genotypic screening. However, much more is to be done and evolving technology supports resolving significant challenges in pharmacology and drug discovery. Technical inadequacy and lacking data reliability may pose severe limitations in compounds discovery and approval by regulatory agencies as the US Food and Drug Administration (FDA). Indeed, clinical trials are founded by safety and efficacy metrics, with pivotal trials (e.g. phase III trials) seeking for evidences to either support or reject the introduction of a new drug onto the market. Clinical review audits comprising of meticulous assessment of bio-clinical documents (Gurevich and Gurevich 2014) are intended to verify both methods and outcomes of clinical trials, along with the reported accuracies; assuring that related documents correctly describe the reported studies. During the process, data quality and its impact on the aftermaths are evaluated. Ultimately, subject eligibility, level of drug compliance, use of concomitant medications, along with both primary and secondary trial endpoints are measured against the importance of the clinical trial, to support submissions to, for instance, either the Biologics License Application (BLA) or the New Drug Application (NDA).

Equally, correctness, reliability, and accuracy drive pharmacology, because the appropriate definition of challenges, controversies and knowledge gaps in drug development result in resilient investigation of the potential of new compounds mitigating pathological signalling cascades (Zhu et al. 2019; Austin et al. 2019; Taquet et al. 2019; Tefera et al. 2019). Addressing phytocompounds activity and the synergetic interaction between the molecules forming a certain complex enables informative decision-making on drug prescription, safety, and efficacy (Thierauch 2011; Mócsai et al. 2014; Vijayakumar et al. 2016; Roy et al. 2017; Sharma et al. 2018); and assistive computer science adds value by increasing accuracy, leveraging both data mining and analysis of large volumes of information.

24.2 Latest Trends in Pharmacology and Fundamentals of Drug Discovery

Pharmacology relies on the correct identification of challenges, controversy and knowledge gap in drug development (Zhu et al. 2019; Austin et al. 2019; Taquet et al. 2019; Tefera et al. 2019). Indeed, the identified limitations open new roads to drug discovery (Pereira 2017a, b; Zhang et al. 2019a, b; Lee et al. 2019; Froux et al. 2019; Vlachodimou et al. 2019), sustained by well-designed drug trials (Fig. 24.1). Drug trial is a fundamental element in bringing new compounds to the market. This is the ultimate step in new therapies' evaluation, which is intended to assure effectiveness and safety, following assessment criteria employed in both basic and translational research (Fig. 24.2); providing qualitative and quantitative information on prescription and adverse effects, tracking comparison drivers as the relative risk reduction (RRR), the absolute risk reduction (ARR), and the numbers needed to treat (NNT) (Friedman et al. 2015a, b, c, d, e, f, g, h, i; Food and Drug Administration 2019b).

Fig. 24.1 FDA Drug Approval Process showing the synergetic correlation between compounds production, development challenges, drug discovery, and clinical trials—source, Food and Drug Administration (2019a)

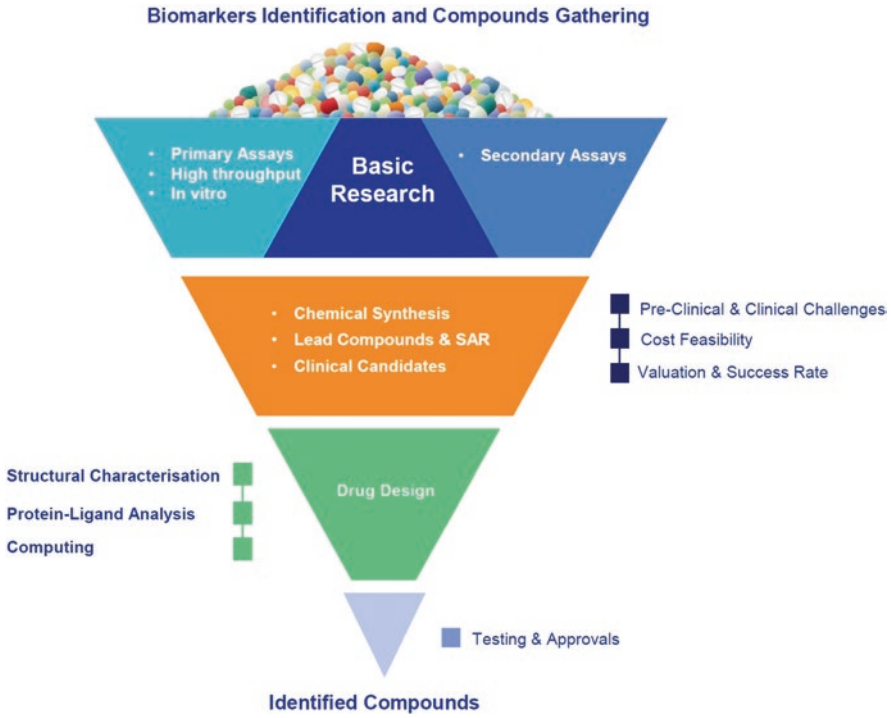

Fig. 24.2 Major elements in drug discovery and new compounds assessment

Public health is critical, with the World Health Organization (WHO) reporting on critical trends on progression of varied pathologies and its impact in socio-economic development. Indeed, in 2005, according to the WHO, neuropathologies resulted in 92 million DALYs (disability-adjusted life years), a measure of disease burden, in terms of reducing life expectancy, with an expected 12% increase due 2030, resulting in these predicted figures peaking at 103 million (WHO 2005, 2012). Additionally, the burden of Alzheimer and other dementias is expected to increase by 66%, from 2005 to 2030 (WHO 2005, 2012). Given the background, progress in pharmacology is benefitting from innovative biotechnology, including genetic engineering and synthetic biology. One example arises from stem cell technology potentialising regenerative medicine, which combined with plant extracts as the 5H-purin-6-amine from *Sedum sarmentosum* can significantly benefit fertility studies. Indeed, 5H-purin-6-amine from *Sedum sarmentosum* extracts modulate increasing spermatogonial stem cell (SSC) action, optimising SSC concentration within germ cells (Jung et al. 2017).

In regenerative treatments, phytocompounds are at the vanguard, with high mobility group box 1 (HMGB1) as an upstream mediator in tissue regeneration. Therefore, optimising and accelerating injury-recovering, enhancing health tissue preservation, and extenuating aging-related tissue impairment. Hence,

pharmacology-based phytocompounds can significantly impact regenerative cell technology, modulating signalling cascades and matching with nanotechnology for precision medicine-driven drug delivery.

Remarkably, the US Food and Drug Administration (FDA) has increasingly accepted genetic engineering and gene-targeting therapies, following risk and safety assessment processes (Food and Drug Administration 2019b, c; Marks 2019). In August 2019, following experimentation with cell lines, the FDA approved the third gene-targeted therapy for cancer patients suffering from genotypic-driven tumour progression. Innovation relates to common variants across different tumour types used as biomarkers for therapeutic prescription, rather than tissue-targeted tumour location. Research on common disease-common variant (CD-CV) is driven by disease occurring in populations that share common alleles (Pereira 2017a), which results in new ways to understand disease progression and initiation, leading to genotype-phenotype-driven treatments. Pharmacology plays here a fundamental role, which combined with genomics (pharmacogenomics) enables better understanding of omics-determined compound activity in both novel therapeutic design and drug repurposing (Pereira 2017a).

24.3 Phytocompound-Based Drug Discovery and Novel Therapeutics

Phytocompounds are becoming central in drug discovery, for displaying a myriad of therapeutic properties (Fig. 24.3), usually low toxicity, and high potential to be combined with nanoparticles enhancing drug delivery and precision medicine. In Parkinson's disease, to mention one example, the therapeutic use of plant secondary metabolites is promising, offering long-term modulation of biomarkers for neurodysfunction (Vijayakumar et al. 2016). Computer science is additionally optimising drug discovery and development methodologies, automating existing processes, increasing models' accuracy, and enriching the achieved results by promoting data mining-based data reliability (Fig. 24.4). One of the distinguishing features of biocomputing is that it is driven by prior knowledge, with distinctive capabilities to correlate and analyse phytocompound properties and interactions (Sharma et al. 2018), at scale, leveraging drug discovery and development.

In nanotechnology, analysis of functional groups found in phytochemicals as potential capping ligands during synthesis of nanoparticles (NPs) could permit controlling the interactions between NPs and the host organism. This would greatly benefit nanodiagnostics and nanomedicine, because the degree of NP-host biological interaction dictates the pathway that nanocompounds would take in the human body and, consequently, the efficacy of nanotherapies. In Abraham et al. (2018), phytochemical-capped silver nanoparticles (AgNPs) interactions with human serum albumin (HSA) were investigated. In that work, curcumin (Cur) and epigallocatechin-3-gallate (EGCG) were found to act as reducing agents, for phytochemical-capped AgNPs that synergistically interact with HSA. The authors show that phytochemical-capped AgNPs-HAS interactions are more significant than citrate-stabilised

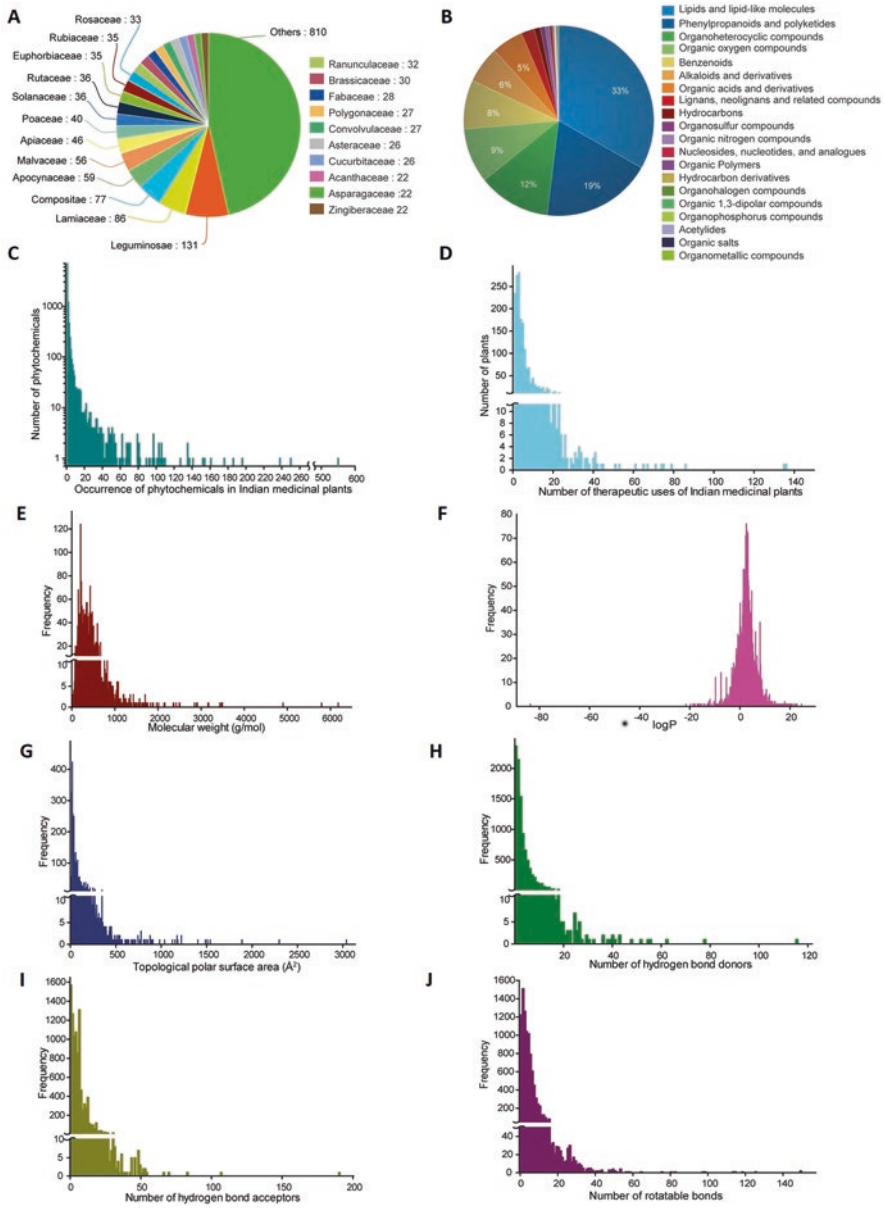

Fig. 24.3 Illustration of plant extract distribution, based on resulting phytocompounds' properties. Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database statistics—source, Mohanraj et al. (2018): (**a**) distribution of 1742 Indian medicinal plants found in the IMPPAT database across different taxonomic families, (**b**) distribution of 9596 IMPPAT phytochemicals across different chemical ClassyFire 50 superclasses, (**c**) plants whose extracts are found in the IMPPAT, (**d**) therapeutic applications of IMPPAT phytocompounds, and (**e**, **j**) molecular weight (g/mol), log P, TPSA (Å^2), number of hydrogen bond donors, number of hydrogen bond acceptors, and number of rotatable bonds of the phytochemicals in the IMPPAT

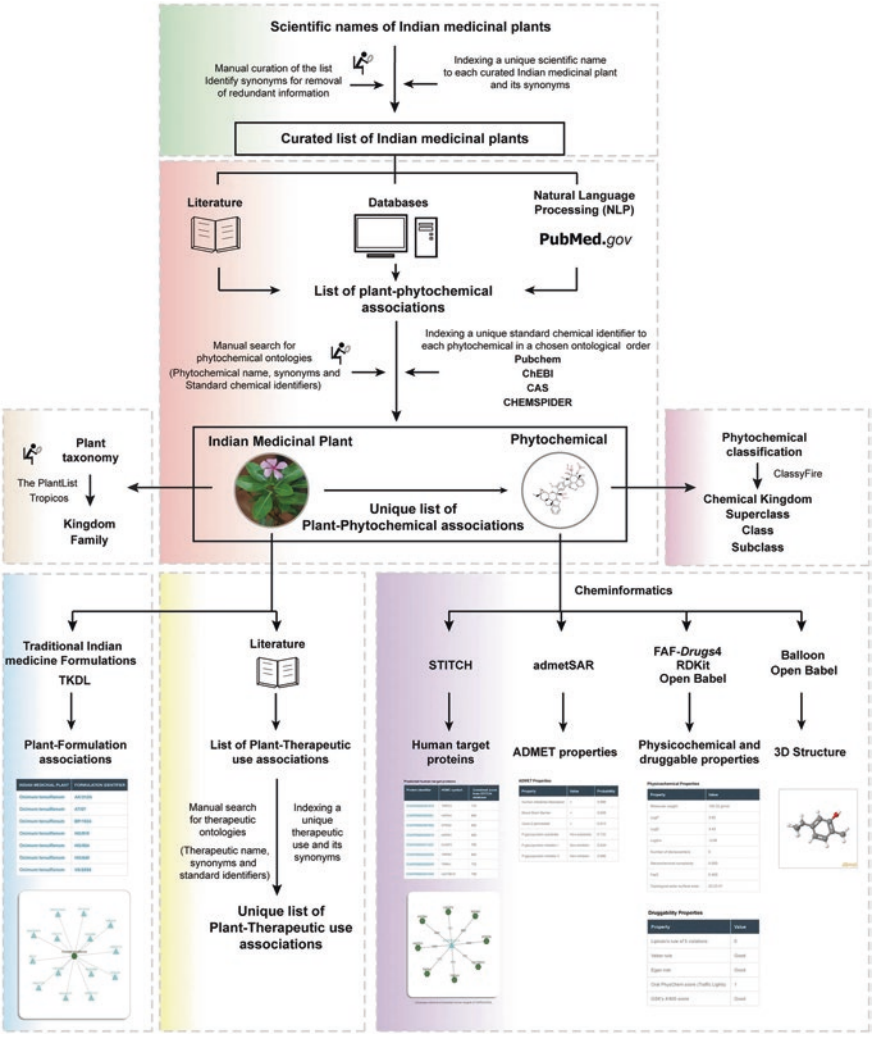

Fig. 24.4 Example of data mining enhancing drug discovery. The diagram shows the IMPPAT database construction pipeline—source, Mohanraj et al. (2018). Data mining enabled automated compilation of a comprehensive list of Indian medicinal plants and plant-formulation associations, using information from various sources including PubMed abstracts and TKDL database. The authors manually annotated the data, to label identified phytochemicals using standard identifiers for building a non-redundant library of phytochemicals. However, we are of the view that artificial intelligence (text mining) could have been used to automate this step. Ultimately, cheminformatics enabled extracting 3D structures, physicochemical properties, drug-ability scores, predicted ADMET properties, and predicted target human proteins of phytochemicals

AgNPs-HAS interactions, with the last showing lower stability, which beneficially preserves the naturally occurring protein conformation. Thus, the authors advocate in favour of selection of phytochemicals driving design and modulation of NP–HSA interactions. Molecular docking is promising, and the technique uniquely possesses a redefining efficacy in diverse applications, by forecasting functionality on the basis of the resulting structural conformation of in-complex molecules that stably bind to each other. It has been used to predict the action of phyllanthus complexes alleviating chronic pain (Chopade et al. 2015) and to analyse *Bacopa monnieri*'s phytocomplexes knocking down pathogenic signalling triggered by *Staphylococcus aureus* (Emran et al. 2015).

In cancer research, molecular docking is envisioned to become an alternative for chemotherapy, radiotherapy, and surgery, mapping protein-phytochemical interactions, to knockdown tumour signalling cascades (Singh et al. 2017; Elengoe and Hamdan 2018). Ultimately, in Choudhary and Singh (2019), molecular docking integrated into a network pharmacology design (Fig. 24.5) contributed with the poly-pharmacological evaluation of anti-epileptic Ayurveda extracts. In that study, signalling cascades resulting from molecular interactions involved in the pathophysiology of epilepsy were examined, with the assistance of in silico docking studies.

24.4 Drug Development in the Age of Digital Transformation

Fast pace continuously changing new technologies are challenging. They require growing technical capabilities, improving infrastructure, specialised human resources, and theoretical background effectively translated into practical results. However, digital transformation brings about increasing potential for discovery and development, which in therapeutics accomplishes myriad of benefits for society (Fig. 24.6). A great deal of academic research in drug discovery involves understanding trends, capturing often subtle phenomena, ultimately productionalising medicinal compounds at scale. Data is the raw material for a significant proportion of drug development activity. Thus, big data governance, correlations, and analytics are fundamental elements promoting biomedical advancement.

For several years, rather centuries, the scientific community found in lacking technology a great limitation for its ideas and endeavours. One single example of how existing open problems can become trivial is the solvability of NP-complete problems, in polynomial time. To date, instances I of polynomial (P), non-polynomial (NP), and NP-complete sets can be verified for decision on suitability of a given candidate for solution, in polynomial time. However, whether NP-complete problems are solvable in polynomial time is still undecidable. A theoretical proof would resolve the question. One remark is that time complexity (O-notation) is key. This is because decision problems that would be potentially solved in exponential time, only, result in a search-time t peaking rapidly, tending to infinity ($t = \lim_{n \to +\infty} f(n) = +\infty$), in a limit sense, with growing instance's characteristic size n. Thus, finding representations P of instances $I \in$ NP-complete is one of the key

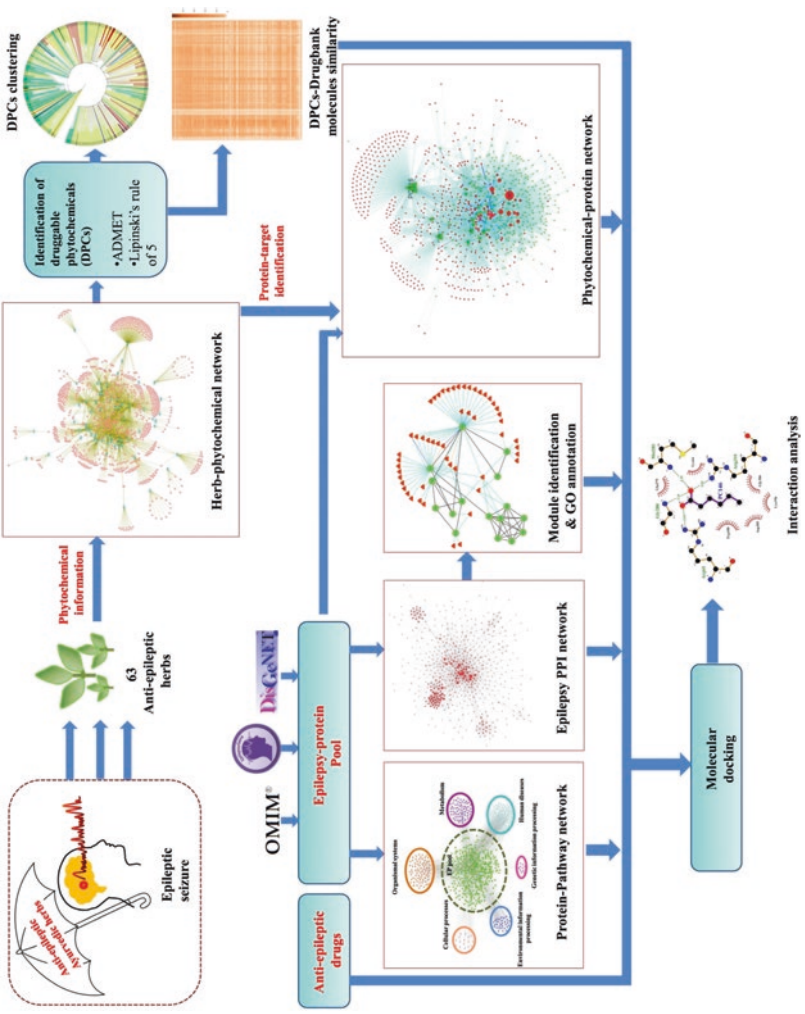

Fig. 24.5 Schematic view of network pharmacology design enriched by molecular docking, for analysing phytocompound interaction-driven modulation of disease pathophysiology—source, Choudhary and Singh (2019)

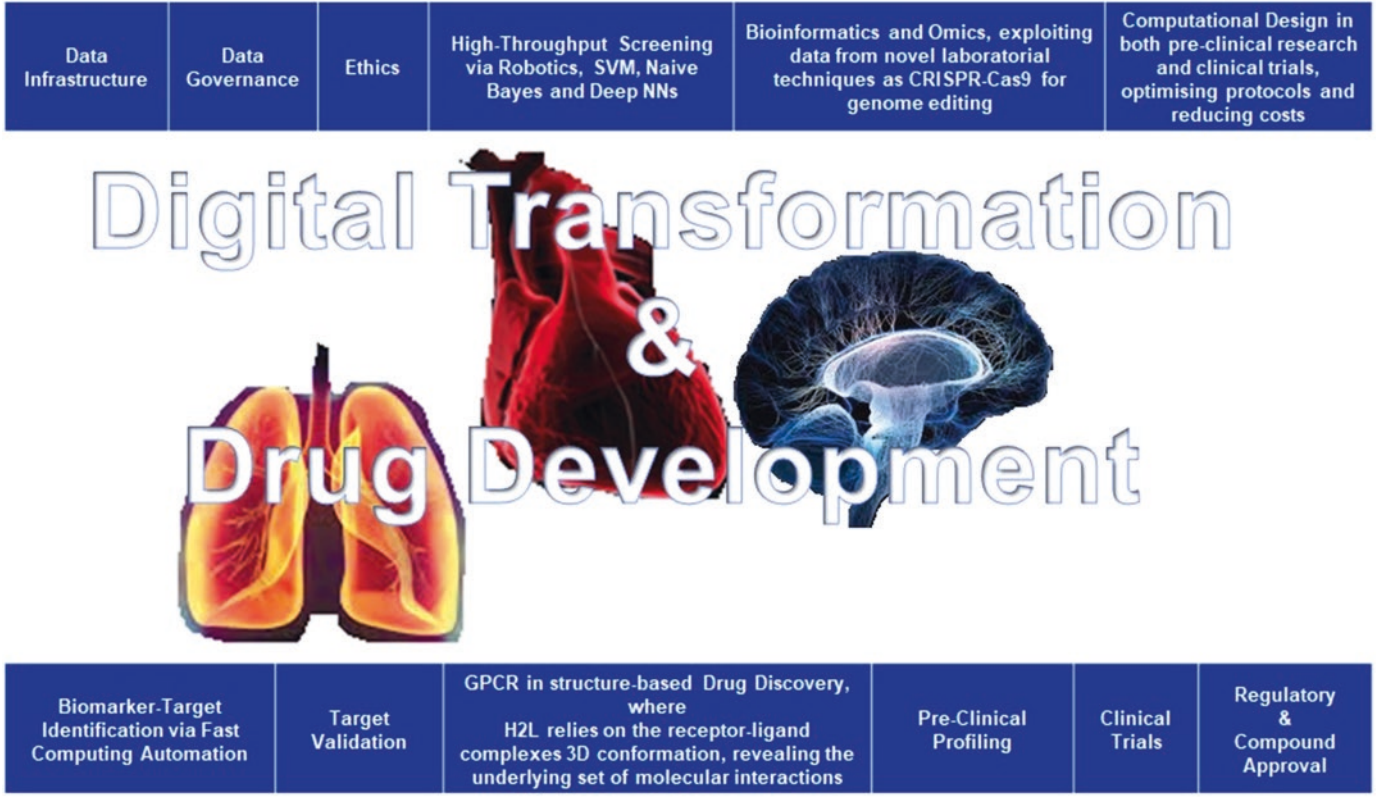

Fig. 24.6 Illustration of the Digital Transformation and Drug Development interplay. Digital transformation may offer different ways to approach clinical research and bio-pharmaceutical discovery and development, better exploring multiple data sources, advancing clinical trial, raising compounds productivity, and improving the quality control of clinical trial and their outcomes

elements forming the field of intractability and combinatorial optimisation (Garey and Johnson 1979; Lima et al. 2005, 2008; NP-Completeness 2008). The Cook-Levin theorem states that the Boolean satisfiability problem is NP-complete, implying that any instance of NP-complete can be reduced in polynomial time by a deterministic Turing machine to Boolean satisfiability. Thus, if a deterministic polynomial time algorithm for solving Boolean satisfiability is found, any NP-complete problem could be solved by a deterministic polynomial time algorithm. Finding such algorithm would represent responding to P = NP, which is among the most significant unsolved problems in computer science. Nevertheless, both advancing computational power and finding innovative ways of processing information would result in restrictions on *t*, to become negligible. Altogether, quantum computing is one example of new technology that could revolutionise experimental mathematics and computing, opening new routes in decidability and NP-completeness.

In drug discovery, digital innovation is intended to promote big data analytics and process automation, leveraging handling cases that rely on large volumes of heterogeneous information, for insights discovery and forecasting, in decision-making. New technologies enable targeting medicinal phenotypes, matching those with pathological genotype-phenotype relations, for either drug discovery or compounds repurposing (Kitano 2002; Lecun et al. 2015; Pereira 2017a; Sharma et al. 2018; Glicksberg et al. 2019; Pereira et al. 2019). Ontologies have been used to characterise molecular compounds, resulting in annotated standardised information systems containing assembles of medicinal compounds, stored in dictionaries (e.g. SNOMED) and semantic networks (e.g. Unified Medical Language System) (Lindberg et al. 1993; Stearns et al. 2001). Small molecules have dominated the pharmaceutical industry, because small molecules development and delivery are simple. However, biologics are gaining space in drug development (e.g. large peptides, recombinant proteins, fusion proteins, antibodies, monoclonal antibodies, nano-bodies, immunotherapeutics, soluble receptors, recombinant DNA, antibody-drug conjugates, and synthetic vaccines), because the therapeutic use of small molecules may pose some adversities—e.g. small molecules can easily function as enzyme inhibitors of satellite signalling cascades and targeting protein-protein interactions-based impairment modulation often depends on the action of protein-based compounds, rather than small molecules (Thierauch 2011; Mócsai et al. 2014). This opens up new prospects for computational design for functionally engineering proteins, targeting desired characteristics, in next-generation therapeutics (Roy et al. 2017).

24.5 Artificial Intelligence in Pharmacology

Biotechnology empowered by cognitive analytics is dictating trends. Artificial Intelligence (AI) is increasingly employed in genotype-phenotype analysis, elucidating compounds' interactions, and tracking diseases' biomarkers. AI techniques enhance conventional systems with the capability of analysing large volumes of distributed data, to elucidate and designate potential ligand-receptor binding

resulting in disease initiation and progression. Myriad of methods can be discussed. Generative Adversarial (Artificial Neural) Networks (GANs) comprise of a generator and a discriminator playing together. The model grows in expertise with the generator increasing ability of creating false patterns, while the discriminator optimises its capability of detecting these fake signals (Fan et al. 2019; Naruse et al. 2019). Deep Learning (DL) consists in multi-layered mathematical structures, often represented via artificial neurons, clustered by specialty. The aim is to break down complex tasks, in a divide and conquer manner (Pereira 2017a). A long-short term memory (LSTM) is a classic example of recurrent neural networks (RNNs) falling into the deep learning category. LSTMs are commonly applied to analyse time series (e.g. video and audio), along with single data points (e.g. images). Another example of deep learning structure is found in convolutional neural networks, which are commonly used in image analysis, being inspired by the functional and structural organisation of neuronal cells that are responsible for deciphering visual inputs. Reinforcement Learning (RL) learns on the basis of performance optimisation (Kaelbling et al. 1996; Pereira 2017a), using a reward-penalty trade-off, as described in behaviourism studies, which is a branch of psychology (Thorndike 1898). The underlying cases can be modelled as a variation of Markovian processes, where the probability $P[S\{t+1\}|S\{t\}]$ of transitioning to state $S\{t+1\}$ depends only on state $S\{t\}$. Indeed, a Markov process (Markov chain) is a tuple (S, P), such that S is a finite set of states and P is the state transition probability matrix. The dynamic is summarised as follows: $[s_0 \rightarrow s_1 \rightarrow s_2 \rightarrow \cdots]$. A Markov decision process (MDP), on the other hand, implies reward and penalty. This is represented by a tuple (S, A, P, γ, R), such that S is a finite set of states, A is a finite set of actions subject to either reward or penalty, P is the state transition probability matrix, $\gamma \in [0, 1]$ is a discount factor, and R: $S \times A \rightarrow R$ is a reward function. MDPs are the mathematical entities that emulate reinforcement learning. Therefore, in a MDP, transitioning to state $S\{t+1\}$ depends on state $S\{t\}$, the action $A\{t\}$, and the reward-penalty trade-off represented via γ and R. The dynamic is summarised as follows: $[s_0\ a_0 \rightarrow s_1 a_1 \rightarrow s_2 a_2 \rightarrow \cdots]$. Optimality is attained by maximising gain. The net discounted reward at time step t is $G\{t\} = [R\{t+1\} + \gamma R\{t+2\} + \cdots]$. The discount factor plays an important role, as $\gamma \approx 0$ represents "myopic" evaluation and $\gamma \approx 1$ represents "far-sighted" evaluation. Indeed, the discount factor prevents cyclic MDPs from enduring infinite reward. The final element in a MDP is the distribution on actions and states $\pi(a|s) = P[A\{t\} = a \mid S\{t\} = s]$, which defines decision on action $A\{t\}$ at state $S\{t\}$. Details on the mathematical formulation of MDPs and their resolution using dynamic programming can be found in Ruszczyński (2010), Sutton and Barto (2012), van Otterlo and Wiering (2012), Bauerle and Riess (2015), and Delgado et al. (2016).

As indicated, artificial intelligence offers assortment of choices on cognitive and associative techniques, from natural language processing (NLP) and text mining for insights discovery (Rajman and Besançon 1998; Kao and Poteet 2007) to hybrid (Zheng et al. 2017) and mixed reality (augmented and virtual reality) for smart assistance and immersive computing (Microsoft 2019; Winkler et al. 2019; Fanini and Cinque 2019). In pharmacology, the value of artificial intelligence and computer

science is incommensurable: (i) computing-driven screening libraries for genotype-phenotype matching in drug discovery and repurposing (Pereira 2017c; Aulner et al. 2019); (ii) recombinant DNA benefitting from genetic engineering design (Adleman 1994; Rothemund et al. 2004; Qian et al. 2011; Gould et al. 2014; Pereira 2017c); (iii) dynamic compound-disease matching (Pereira 2017c; Romano and Tatonetti 2019; Ekins et al. 2019); (iv) computational pattern recognition and forecasting, targeting chemical compounds for drug repurposing, and foreseeing individual response to new treatments (Pereira 2017a); and (v) polyphenol-protein interactome unveiled via data mining, correlation, and database annotation (Lacroix et al. 2018).

GANs (Kadurin et al. 2017; Putin et al. 2018a, b; Chan et al. 2019; Basile et al. 2019), DL (Aliper et al. 2016; Chen et al. 2018; Zhavoronkov et al. 2019; Batool et al. 2019), and RL (Putin et al. 2018a, b) are paving the way for innovative drug

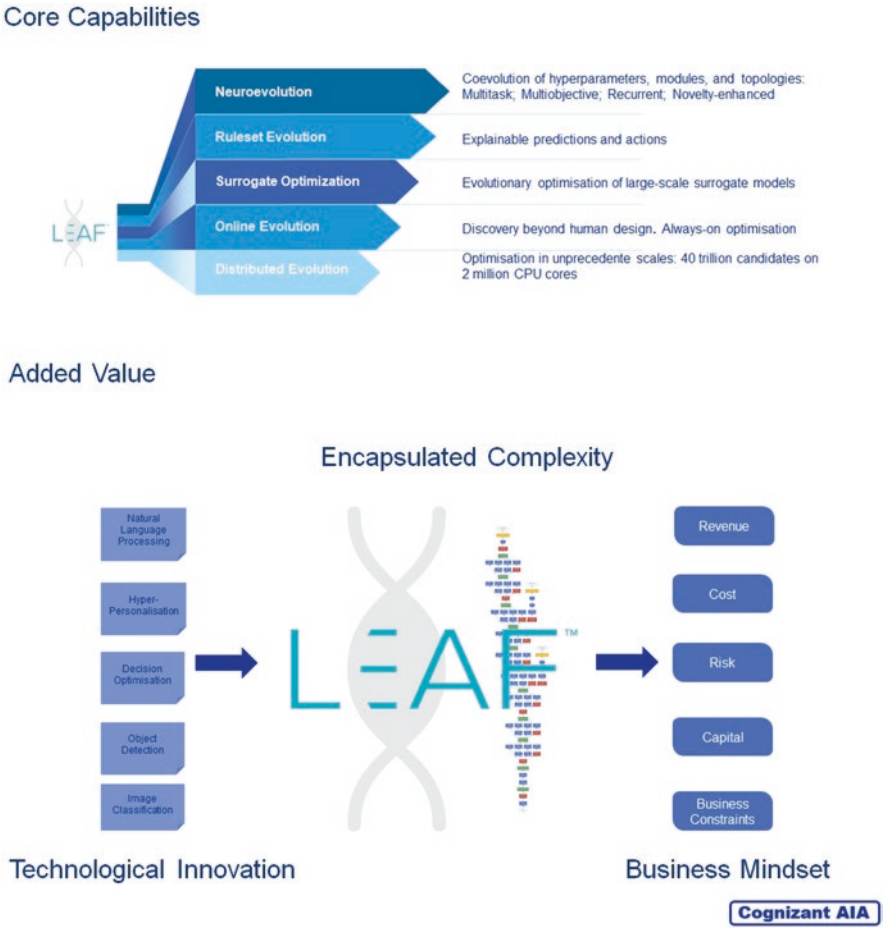

Fig. 24.7 Illustration of LEAF capabilities, indicating both innovative pathways and business value

discovery, *de novo*-based design of new molecules, prediction of pharmacological responses in drug repurposing, structure-based molecular engineering, and interventional pharmacology enriched by word embedding and attention mechanisms to extract drug-adverse event (AE) relationships. One example of usage of deep learning technology is found among algorithms employed by Cognizant AIA teams, which are investing in innovation (Fig. 24.7), with a focus on deep learning technology (Cognizant 2019; Liang et al. 2019). The main platform is named Learning Evolutionary Algorithm Framework (LEAF™). LEAF comprises of two major schemes—evolutionary neural networks (LEAF-ENN) and evolutionary surrogate-assisted prescription (LEAF-ESP). The core platform is intended to optimise hyperparametrisation, including decision on the size of artificial neural networks. LEAF-ESP is based on state-of-the-art cognitive AI capabilities, optimising prescription on actions that result in maximum gain. The underlying applications are of great interest for the industry, broad scientific community with varied solvers employed in the field of combinatorial optimisation—e.g. graph theory, satisfiability, and Markov chain techniques searching for optimal closed paths (the action of walking to consecutive sites, in a minimally costly order, in a path that begins and ends at the same site) that resolve the salesperson problem (Lima et al. 2005, 2008; Tang and Miller-Hooks 2005). Artificial intelligence is revolutionising biomedical research.

24.6 Conclusions

It is becoming increasingly difficult to envision a world without the added value of technology. However, sustainability is among the major challenges modern society faces, though, because increasing technology is a two-way road, with technological inadequacies often posing threats to safety (e.g. increasing computational power facilitating cybercrime) and to the preservation of ecosystems (e.g. high-speed jet engines increasing carbon emissions and, consequently, atmospheric concentrations of carbon dioxide). Research is evolving towards pushing the limits, to mitigate these drawbacks. Nonetheless, we might aim at compromising, because in general lines, evidences show that system sustainability often relies on equilibrium states. Therefore, this chapter sets out to discuss the value of using technology and innovative computing techniques, to leverage drug development. Our purpose was to examine the current literature, reporting on biomarker assessment, drug discovery stages, the importance of assessing phytocompound activity in disease progression, and on how innovation, particularly artificial intelligence subfields, positively influences research in pharmacology and medicinal compound production, at scale. The trade-off production possibility frontier (PPF) was implicitly discussed when FDA standards on safety and efficacy were conversed. Thus, we concluded by addressing fundamental methodologies for insight discovery, forecasting, and decision-making in big data analytics, endowing biomedical research with cognitive power (e.g. biotechnology empowered by cognitive analytics), which is used to analyse genotype-phenotype pairs, target compounds' interactions, and track diseases' biomarkers. We are of the view that it is proven that drug development can undoubtedly benefit

from computation. However, the question raised by this study is how optimal design and technological adequacy would ultimately define the success of drug development, resulting in an increasing number of compounds being either discovered or repurposed, with regulatory processes being resolved in optimal timescales.

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Computational Methods Used in Phytocompound-Based Drug Discovery

25

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Abstract

Phytocompounds are gaining popularity, due to lesser toxicity, greater bioavailability and high chemodiversity. Phytocompounds are evolving as new leads for the development of a novel drug. Phytocompounds exhibit a wide range of biological properties, and are being used as antioxidants, immunomodulatory, antimicrobial, cardiovascular, and anticancer drugs. However, their identification is still relatively limited. The major hurdle in the discovery of an efficient phytocompound is a complete dependence on time-consuming in vitro and in vivo screening systems. Alternatively, the computational drug discovery approach of using an online database and bioinformatics tools would be a cost-effective and time-saving option. Recent advancements in computational and structural biology have attributed the three-dimensional (3D) structure to many natural drug-like compounds and disease-related target macromolecules, and have been stored into renowned databases like Protein Data Bank (RCSB-PDB), DrugBank, and ZINC. The drug discovery filed is swiftly advancing with bioinformatics applications, and the availability of digitalized molecular data is paving new avenues in Computer-aided drug discovery (CADD) area. Virtual library screening (VLS), a widely recognized technique due to it being cost-effective, time-saving, and less laborious, evaluates drug candidates using computational tools, such as AutoDock Vina, GOLD, and Glide. This chapter provides comprehensive information about the available biological databases and bioinformatics tools that are useful for the analysis of plant-derived bioactive compounds and their molecular interactions in diseases. Applications of advanced bioinformatics tools and methods for designing, optimization, and high-throughput screening of phytocompounds are detailed. In addition, it emphasizes the advantages, limitations, challenges, and future perspectives of the computational approaches in analyzing phytocompound interactions.

Keywords

Phytocompounds · Drug discovery · Bioinformatics · Databank · Computational drug designing

25.1 Introduction

Nowadays, the worldwide population is mainly depending on medications derived from herbal sources to overcome many health issues. In spite of the fact that drug discovery has been driven by a variety of innovation stages by natural product specialists, the drug development remains a protracted procedure with a low rate of progress and investment. With the huge number of plant species on earth, we are enriched with a tremendous abundance of therapeutic cures from Mother Nature (Pan et al. 2013). Natural items and their subsidiaries speak to over half of the considerable number of medications in the current therapeutics (Pan et al. 2013). When all things are considered, it takes around 10 to 15 years for a recently integrated compound to turn into an attractive therapeutic agent, and the expense incurred for it in 2006 was roughly €1 billion (Pan et al. 2013; Barden and Weaver 2010).

Phyto is the Greek word for plants, and phytochemicals are chemical compounds that occur naturally in plants (Yahia et al. 2013). Plants produce phytochemicals so as to ensure themselves against natural dangers like predator insects, pollution, and disease (Yahia et al. 2013). Phytocompounds have been recognized as a promising focus of research in the journey for new pharmaceutical compounds as they exhibit chemical structure diversity with biological properties (Chen et al. 2013). In general, phytochemicals are nonessential supplements of plant nourishments that are not required to support life. However, a developing collection of proof recommends phytochemicals as valuable resources to support our health (Chen et al. 2013; Swamy et al. 2016). There are several numbers of phytochemicals belonging to different chemical classes, such as polyphenols, flavonoids, saponins, terpenoids, etc., that are being reported to possess numerous pharmacological properties (Goyal et al. 2013). Some of the important pharmacological properties include antimicrobial, antioxidant, anticancer, anti-inflammatory, and other health-promoting properties. Dietary supplements rich in antioxidants significantly have medical advantages (Goyal et al. 2013). Antioxidants protect our cells from free radicals and reduce risks of developing certain types of cancer (Goyal et al. 2013; Ham et al. 2013). Although evidence is still being gathered, research findings do suggest that phytochemicals can protect us from a variety of diseases (Ham et al. 2013). For example, previous researches have shown that plant-based foods rich in flavonoids can reduce the mortality rate up to 25% and decrease the instances of myocardial infarction (Fraga et al. 2019). There are a few reasons why phytochemicals may shield us from malignancy and cardiovascular illness. One may be the fact that they tend to inhibit cell proliferation and angiogenesis, i.e., the growth of new blood vessels, which are both hallmarks of cancers (Noor et al. 2014). Also, phytochemicals regulate nitric oxide, relax blood vessels, and therefore increase blood flow rate (Noor et al. 2014; Rai et al. 2014).

The process of novel drug discovery and development is generally recognized to be very time-consuming, risky, and expensive (Vulpetti et al. 2012). Typical drug discovery and development cycle, ideas to showcase, take around 14 years, and the

cost required for the same ranges from 0.8 to 1.0 billion USD (Ou-Yang et al. 2012; Vulpetti et al. 2012; Mohs and Greig 2017). Rapid developments in combinatorial chemistry and high-throughput screening technologies have given a domain to facilitate the drug discovery process. Further, research advancements have empowered gigantic libraries of compounds to be screened and explored in brief time to develop novel lead molecules (Lahana 1999; Varnek et al. 2004; Ou-Yang et al. 2012). In spite of new medication, advancements have developed, and the yield is not emphatically relating to the speculation on the low proficiency and high disappointment rate in drug discovery (Shekhar 2008; Ou-Yang et al. 2012). Subsequently, different methodologies have been created to abbreviate the research cycle and lessen the cost and danger of disappointments for drug discovery (Ou-Yang et al. 2012). Computer-aided drug discovery (CADD) is a standout among the best strategies for achieving these objectives (Veselovsky and Ivanov 2003, Ou-Yang et al. 2012). Computational drug discovery is a compelling technique for quickening and streamlining drug revelation and the advancement process (Veselovsky and Ivanov 2003; Ou-Yang et al. 2012; Elengoe and Hamdan 2018). Due to increased accessibility of biological macromolecules from nature, small molecule data and their computational medication approaches have been stretched out and are comprehensively connected to each phase involved in the drug discovery. The computational approached has significantly improved the drug discovery process from target identification to approval stage (Veselovsky and Ivanov 2003; Ou-Yang et al. 2012; Alimi et al. 2013). Over the previous decades, computational drug discovery techniques, for example, molecular docking, pharmacophore modeling and mapping, de novo design, molecular similarity calculation, and sequence-based virtual screening, have been greatly improved (Ou-Yang et al. 2012; Rifaioglu et al. 2018). In the post-genomic era, the exploration of computational instruments/tools, profiling of macromolecules, and molecular data processing have significantly increased (Jorgensen 2004; Shekhar 2008; Zhang 2011; Xiang et al. 2012). These recorded macromolecular data can be even connected to preclinical preliminary studies. In this way, computational tools can enormously improve and enhance the pipeline process of drug discovery (Ou-Yang et al. 2012). Further, the utilization of computational tools may reduce the expense involved in drug discovery up to half (Tan et al. 2010; Ou-Yang et al. 2012). Virtual library screening (VLS) uses computer-based methods to discover new ligands on the basis of biological structures. The predominant system for the identification of new lead compounds in drug discovery is the physical screening of enormous libraries of synthetic and natural agents via high-throughput screening (Shoichet 2004). An alternative approach, known as virtual screening, computationally screens large libraries of chemicals for leads that complement targets of known structure and experimentally test those leads predicted to bind well.

The current chapter briefly introduce various classes of phytocompounds and their medicinal importance. This chapter mainly focused on the summarizing the computational techniques ranging from ab initio to high-throughput drug discovery methods, associated free and commercial software, as well as their applicability with respect to plant-based drug molecule development. Additionally, this chapter

elaborated the powerful approaches like structure-based drug design (SBDD) and ligand-based drug discovery methods and applications in the context of plant-based curing compounds.

25.2 Classes of Phytocompounds and Their Medicinal Importance

For many generations, people are using plants and plant-based products as natural therapeutics. The innate defense mechanism of the plants contributes to the natural healing capacity against various human health issues (Sudipta et al. 2012; Swamy et al. 2017; Mohanty et al. 2017). Various chemical substances of a plant contribute to different color, texture, and aroma in different parts, including leaves, fruits, and flowers. Further, these compounds have evolved to be a part of the plant defense mechanism to protect from bacterial, viral, and fungal attacks and other environmental challenges. On the basis of the chemical structure and presence of functional groups, phytochemical substances are broadly categorized into several major classes like alkaloids, terpenoids, saponins, polyphenols, etc. Further, various different classes of the phytochemicals are grouped according to their chemical properties and structures. The main three phytochemical classes are briefly described hereunder.

25.2.1 Alkaloids

Alkaloids are the class of naturally occurring chemical constituents composed of heterocyclic nitrogen-containing bases, predominantly found in plants, chiefly in certain angiosperms. They show diverse physiological effects on animals, including humans (Hussain et al. 2018). Morphine is the first alkaloid isolated from the plant opium in the nineteenth century (Lesch 1981). Some of the other well-known alkaloids include piperine, quinine, caffeine, nicotine, and ephedrine (Bulduk et al. 2015). More than 3000 types of alkaloids are being identified from various plant species. Some of the plant families that are rich in alkaloids include Solanaceae, Ranunculaceae, and Amaryllidaceae. Alkaloids are further classified into pyrrolidines, pyridines, indoles, quinolines, isoquinolines, and steroids (Cushnie et al. 2014). Alkaloids exert diverse medicinal properties, for instance, morphine is the strong narcotic used in pain relief. Likewise, codeine, the methyl ether derivative of morphine, is used as a potential analgesic agent. Similarly, indoles are effective for hypertension-related problems. Quinines are useful against malaria, while quinidine acts as anti-arrhythmic. Many alkaloids are known for strong antimicrobial activity, especially antibacterial activity. Quinine proved to be a potential antimalarial drug; sanguinarine, an alkaloid derived from *Sanguinaria canadensis*, is the popular antibacterial agent. The combination of sanguinarine with EDTA and vancomycin was shown effective against both gram-negative and gram-positive bacteria (Hamoud et al. 2014). Also, when tested for the anticancer properties, sanguinarine was effective in inhibiting cervical cancer cells via inducing apoptosis mediated by the

inhibition of inflammatory cytokines (Xu et al. 2012). Likewise, alkaloids exhibit a diverse range of therapeutic activities including local anesthesia, respiratory and muscle relaxation, cardiac stimulation, vasoconstriction, hypertensive and hypotensive properties, and antineoplastic activities. Further, alkaloids are proved to be very effective against several neurodegenerative disorders including Huntington disease, Alzheimer's disease, Parkinson's disease, epilepsy, schizophrenia, and stroke. Alkaloids, such as caffeine, vinblastine, ephedrine, piperine, etc., are being used widely in the treatments of numerous diseases (Othman et al. 2019; Hussain et al. 2018). Several drugs, such as vincristine and vinblastine isolated from *Catharanthus roseus* and *camptothecin extracted from Camptotheca acuminata*, are being used for the treatment of cancers (Torquato et al. 2017).

25.2.2 Terpenoids

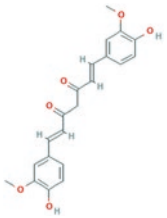
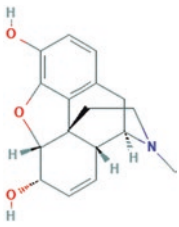
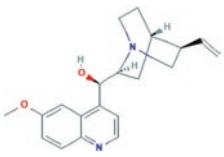
Terpenoids include a structurally varied group of natural products having several potential medical applications. They are widely used as natural flavors, medicines, and nutraceutical agents. Even today, the majority of them are obtained mainly from plant sources. Generally, plants produce terpenes for self-defense to protect from microorganisms like bacteria, fungi, and viruses. Terpenoids are the largest group of phytocompounds and mostly occur in plants in the form of lipids. Terpenes have multi-cyclic ring structures derived from five-carbon isoprene units (de las Heras et al. 2003). Terpenes are categorized into many types based on the number of isoprene units in the molecule. For instance, hemiterpenes contain a single isoprene, unit and monoterpenes possess two isoprene units. Some examples of hemiterpenes include oxygen-comprising derivatives, namely, isovaleric acid occurring in the plant *Vaccinium myrtillus* and angelic acid found in *Angelica archangelica* (Hussein and El-Anssary 2018). Monoterpenes and diterpenes are abundantly found in many essential oil-bearing plants belonging to the families of Lamiaceae, Rutaceae, Pinaceae, and Apiaceae. The well-known monoterpenes of volatile secretions of most plants include turpentine, menthol, carvone, linalool, geraniol, limonene, eucalyptol, and camphor. Most of these volatile compounds possess numerous medicinal applications. For instance, menthol and camphor are used as counterirritant, anti-itching, and analgesic agents. Several monoterpenes are being utilized as anthelmintic agents. Diterpenes, such as retinol (vitamin A), cafestol, and phytol, are composed of four isoprene units. Triterpenes, consisting of six isoprene units, constitute a major share of the lipid constituents of all plants. Some of them include squalene, β-amyrin, lupeol, epifriedelinol, methyl oleanolate, etc. and possess several medicinal importance. Sesquiterpenes, such as patchouli alcohol, bisabolol, farnesol, caryophyllene, etc., have three isoprene units and exhibit several pharmacological properties including antimicrobial and anti-protozoan activities. The anticancer compound Taxol is obtained from plant sources and is a terpene (Zengin and Baysal 2014; Rao et al. 2010; Hussein and El-Anssary 2018).

25.2.3 Polyphenols

Polyphenols constitute a large proportion of phytocompounds and are profoundly available in various edible parts of plants like nuts, fruits, seeds, stems, and flowers. Phenolics are composed of hydroxyl groups bound to aromatic hydrocarbon. To date, more than 8000 phenolic compounds are being identified. They are the most prevalent secondary metabolites in many herbs and contribute considerably to the taste, color, and flavor. Foodstuffs and beverages rich in polyphenols significantly reduce the threat of noncommunicable illnesses. Tea and coffee are the usual phenolic drinks of our day to day life. Basically, phenolics provide defensive responses and possess superior antioxidant and anti-inflammatory properties. Based on the biological activity, structure, and function, they are classified into flavonoids and non-flavonoids (Cutrim and Cortez 2018). Some of the phenolic compounds of pharmacological importance include gallic acid, eugenol, genistein, daidzein, ellagic acid, coumarin, etc. (Hussein and El-Anssary 2018). The leaf and bark extracts of *Duguetia furfuracea* contain phenolic compounds like gallic acid, ellagic acid, catechin, quercetin, kaempferol, etc. and are reported to exhibit potent antioxidative, antimicrobial, anti-inflammatory, and anticancer properties (Pinho et al. 2016). Flavonoids share a large proportion of phenols and are found extensively in plants. They help in coloring of flowers, buds, and shoots. They are abundantly found in vegetables and fruits. Flavonoids are abundantly reported in these plants, namely, *Glycyrrhiza glabra*, *Chamaemelum nobile*, *Ginkgo biloba*, *Betula pendula*, *Calendula officinalis*, *Sambucus nigra*, *Equisetum ramosissimum*, *Tilia cordata*, *Leonurus cardiaca*, *Passiflora edulis*, and more. Flavonoids are further divided into anthoxanthins and anthocyanins. Anthocyanins are reported to possess antimicrobial, antioxidant, anticancer, anti-inflammatory, and anti-neurodegenerative activities (Khoo et al. 2017). Some of the highly valued flavonoids include quercetin, apigenin, luteolin, and chrysin. Some of them are reported to occur commonly in our diet (Liu et al. 2010; Hussein and El-Anssary 2018). Studies have proved that the plant flavonoid drug colchicine acts as a native inhibitor in cancer cells through binding on active sites of P-glycoprotein and ATPase enzymes (Silva et al. 2014). Other non-flavonoid phenols include tannins, phenolic acid, coumarins, and stilbenoids. Tannins are water-soluble polyphenols occurring in several plant foods. They readily bind to proteins and precipitate them. Thus, they have a significant role in influencing the nutritive value of various foodstuffs when eaten by humans. Tannins are phenolic acid polymers with high molecular weight and exist in two different forms: condensed and hydrolyzable tannins. Generally, tannins are found in fruits such as blueberry, persimmon, and grapes as well as other plant sources like sorghum, corn, and legumes (Tsao 2010). *Geranium robertianum*, *Geranium maculatum*, *Quercus alba*, *Punica granatum*, *Filipendula ulmaria*, *Camellia sinensis*, *Hamamelis virginiana*, *Vaccinium oxycoccos*, etc. are some plants where tannins are abundantly found. Coumarins, such as umbelliferone, aesculetin, scopoletin, etc., are reported from plants, such as *Dipteryx odorata*, *Atropa belladonna*, *Datura stramonium*, *Daphne mezereum*, *Ruta graveolens*, and *Aesculus hippocastanum* (Hussein and El-Anssary 2018). Phenolic acids are widely spread in plants. Phenols

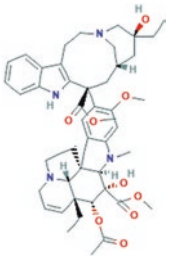
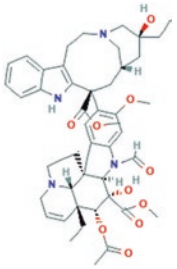
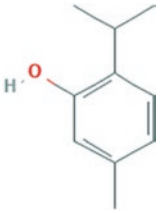
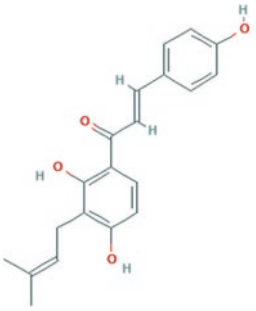
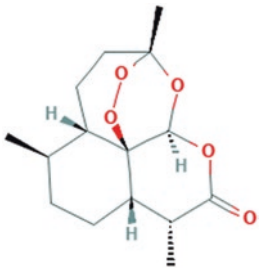
containing the carboxylic acid group designate the name to these compounds. Structurally phenolic acids possess two different carbon alignments, namely, hydroxybenzoic and hydroxycinnamic forms (Wu et al. 2017). Major antioxidants like chlorogenic acid, cyanidin-3-glucoside, and epicatechin isolated from *Melaleuca leucadendron*, *Lagerstroemia indica*, and *Eucalyptus robusta* are widely used in the treatment of chronic diseases, such as cardiovascular diseases, cancers, and Alzheimer's disease (Zhang et al. 2015). Stilbenes are a small group of plant compounds that occur majorly in the heartwood of plane trees belonging to genera *Pinus*, *Eucalyptus*, and *Madura.* Resveratrol (a *para*-hydroxylated compound) is the most widespread stilbene in nature. Resveratrol possesses estrogen-like activity and occurs in *Picea*, *Pinus*, the Fabaceae, Myrtaceae, and Vitaceae. Both non-flavonoid phenols, i.e., coumarins and stilbenoids occurring in fruits and leaves, are reported to have superior antimicrobial and antioxidant activities (Tsao 2010; Hussein and El-Anssary 2018). At present, several phytocompound drugs are commercially available; few of the phytocompound drug's structure and medicinal properties are listed in Table 25.1.

Table 25.1 Commercially available phytocompound drugs their structure and medicinal properties

Compound	Structure	Medicinal properties
Curcumin		Antibacterial, anticancer, antidiabetic, anti-inflammatory
Morphine		Analgesic, pain reliever
Quinine		Antibacterial, antimalarial

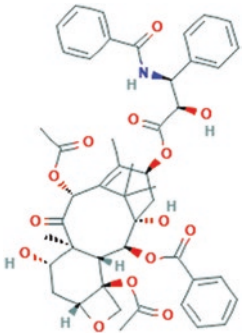
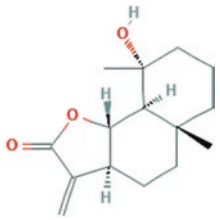
(continued)

Table 25.1 (continued)

Compound	Structure	Medicinal properties
Vinblastine		Anticancer
Vincristine		Anticancer, antitumor
Thymol		Antifungal, antibacterial, dental care
Isobavachalcone		Antiplatelet, antioxidative, antitumor, neuroprotective, anti-inflammatory
Artemisinin		Antimalarial, antibacterial, autoimmune diseases, anticancer

(continued)

Table 25.1 (continued)

Compound	Structure	Medicinal properties
Taxol		Anticancer, antineoplastic
Arbusculin		Melanin inhibitor

25.3 Computational Approaches to Develop Phytocompounds as Drugs

Phytocompounds have several advantages over chemical entities like low cost, lower side effects, and higher bioavailability (Ferreira et al. 2015). In specific, computational approaches help us to explore pharmacokinetic properties, i.e., absorption, metabolism, distribution, excretion, and lethality, and pharmacodynamics data, i.e., affinity, potency, efficiency, and selectivity (Lipinski et al. 1997; Sarvagalla et al. 2019). As most of the modern medicines are derived from plant sources, many phytocompounds are being explored for their pharmacological properties. Computer-aided drug discovery (CADD) is one of the advanced tools in recent times that has certainly increased the effectiveness of the drug discovery process (Scotti et al. 2018). In addition, other cutting-edge technologies, such as virtual library screening (VLS), ligand-based drug discovery (LBDD), and structure-based drug design (SBDD), have further complemented the drug invention. Several of the commercial and free tools, such as AutoDock, Schrodinger suite, Discovery Studio, FlexX, and many more, are being used in CADD to identify the novel inhibitors for numerous targets. At present, researchers are increasingly using the molecular modeling method as a powerful medicinal chemistry tool to study structure-activity relationships (Sarvagalla et al. 2019; Ferreira et al. 2015).

Various databases like Alkamid, Asian anticancer material, Chem-TCM, and CHMIS have incorporated vast information about regional medicinal plants and

useful metabolites that cure numerous human diseases. Recent studies emphasized the role of in silico developed plant-based drugs for various cancers and inflammatory and microbial infections (Tahlan et al. 2019). Latter literature from various studies elucidated the implications of bioinformatics approach in kinase-dependent inhibitor discovery (Kalra et al. 2017). The state-of-the-art computational methods useful for phytocompounds-based drug discovery are elaborated hereunder.

25.3.1 Ligand-Based Drug Design

Ligand-based drug designing or ligand-based drug discovery (LBDD) is one of the renowned high-throughput screening (HTS) in silico drug discovery method. LBDD is also called the indirect drug discovery method, which is mainly based on the known properties of a ligand, and it helps to search for the suitable receptor. Though, numerous new phytocompounds are characterized based on their medicinal properties like antimicrobial, anticancer, antioxidant, etc.; however, the precise target knowledge is generally unavailable in major cases. LBDD is the tailor-fit method for phytochemical druggability evaluation since it depends on the prior knowledge of renowned ligands pharmacophores (Aparoy et al. 2012). Figure 25.1 shows the schematic representation to explain the step by step guide for the ligand-based drug

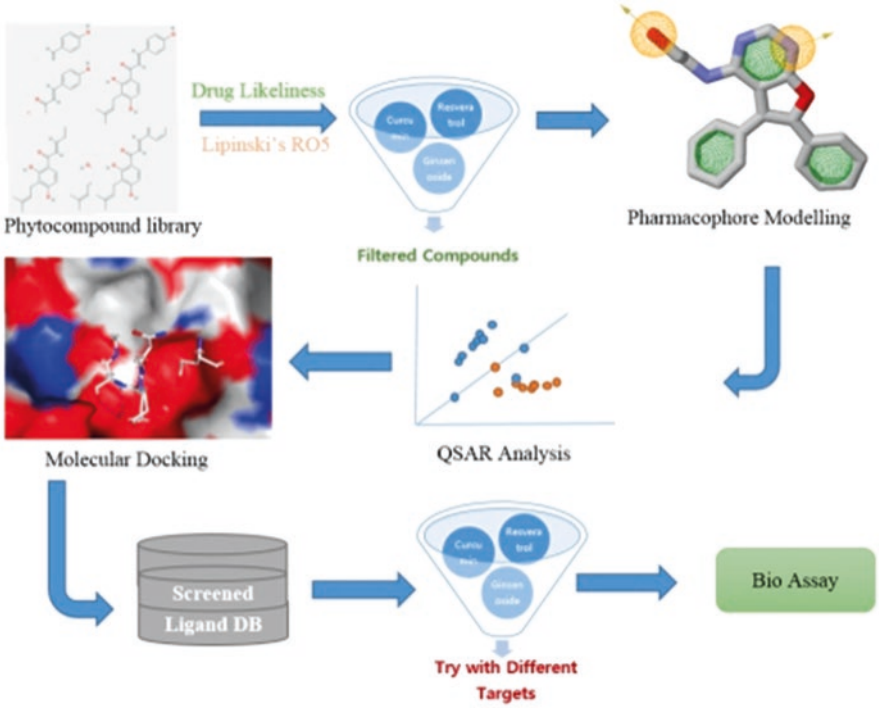


Fig. 25.1 Schematic representation of ligand-based drug discovery for phytocompounds

discovery method, right from ligand selection, optimization, and screening by using the computational method like molecular docking.

The LBDD process starts with the consideration of the medicinal property of a natural compound, and then followed by the three-dimensional structure of an entity obtained through technologies like nuclear magnetic resonance (NMR) and X-ray crystallography (XRD) (Ferreira et al. 2015). The selected structure will be aligned to establish structures from the library or database to yield the pharmacophore knowledge of the compound. Together with the usage of pharmacophore properties and molecular descriptors, new ligands are developed by adding the required functional groups (Prasad et al. 2013). The molecular descriptors explain the shape, bond, charge, and atomic coordinates of the ligand, which are calculated using the quantitative structure-activity relationship (QSAR) methods, including the comparative molecular field analysis (CoMFA). There are numerous online and standalone tools that are available for predicting the drug likeliness of the phytocompounds such as BREED distributed by Schrodinger for ligand design, MedChem Studio for predicting pharmacokinetic parameters, ADMET (absorption, distribution, metabolism, excretion, and toxicity) in ligand-based design, and Leadgrow for the Lipinski rule screening to generate combinatorial library. Additionally, commercial software such as LigandScout and MOE (Molecular Operating Environment) helps in predicting the ligand descriptors like topology, pharmacophore details for improving the efficacy and to understand the receptor-ligand binding sites (Acharya et al. 2011).

The ligand-based drug discovery model is well adopted in anticancer drug discovery. Potential inhibitors were discovered for proto-oncogenes like Bcl2 and P53 and inflammatory agents like TNF-alpha, IL-1, and IKKB using the computational drug designing techniques. Recent studies attributed the implication of in silico screening for aromatase inhibitors the key targets in cancer therapy (De et al. 2018). Further, in another recent study, ligand-based pharmacophore modeling approach was used to discover the inhibitors for the metastatic agent CXC chemokine receptor 2; backbones of known drugs were used to model the pharmacophore in order to get appropriate candidates (Che et al. 2018). On the other hand, recent studies have shown plant-derived compounds that exert anticancer activity through acting on various targets: camptothecin antagonizes the topoisomerase I, bruceantin inhibits peptidyl transferase activity, and combretastatin destabilizes the microtubules (Lichota and Gwozdzinski 2018). The above contexts suggesting the ligand-based approaches combined with pharmacophore modeling help in exploring the novel plant-derived inhibitors and in enhancing the potentiality of the compound.

25.3.2 Structure-Based Drug Design

Structure-based drug design (SBDD) greatly depends on the three-dimensional structure of the target molecule. Figure 25.2 shows the strategy for phytocompound drug discovery using structure-based drug design method. Predicting the novel receptor-ligand interaction is having greater importance in the drug discovery

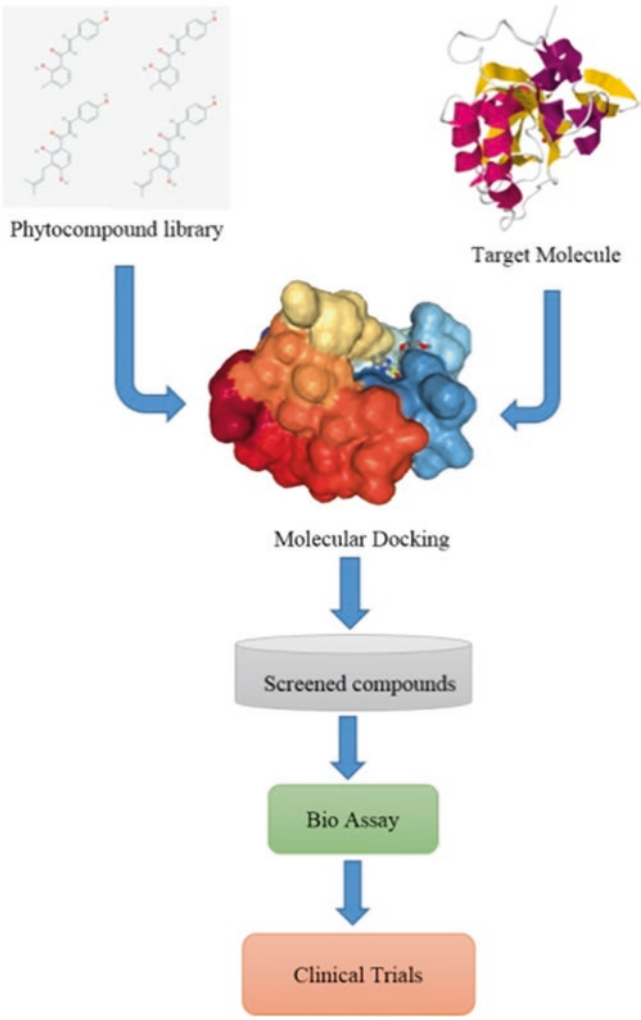


Fig. 25.2 Strategy for phytocompound drug discovery using structure-based drug design approach

industry. Finding an appropriate target structure is the key to the success of SBDD. The cutting-edge technologies like X-ray crystallography and NMR are used to extract the structural information of the targets. The molecules with unknown structure will be modeled using the computational approach like homology modeling (Schmidt et al. 2014). The macromolecules with validated structures enable a meticulous inspection of the binding site topology, i.e., the occurrence of cavities, clefts, sub-pockets, and electrostatic properties. The currently available SBDD techniques allow ligands to be designed with required characteristics for an effective target receptor modulation. The selective modulation by elevated affinity ligands of a validated drug target interferes with explicit cellular procedures and eventually

results in the anticipated beneficial pharmacological impacts (Onodera et al. 2007). The workflow of SBDD involves different levels starting from the target selection, lead optimization, library preparation, and evaluation through docking. Also, it includes the post-processing of a successful phytocompound until the clinical trial level (Shanmugam and Jeon 2017).

Implementing SBDD to phytocompound drug discovery is an efficient method to unveil the potentiality of natural ligands. The process of SBDD for phytochemicals includes similar steps involved for chemical drug design; however, the validation of natural ligands for drug likeness is substantial. Once the target is selected, the ligand library containing phytocompounds will be prepared to dock the ligands with a target for further evaluation. The scoring method will be used to scrutinize the best binding compound based on various properties like efficacy, binding distance, and binding energy (ΔG) (Aamir et al. 2018). The receptor-ligand complex is helpful in unveiling the efficacy of the phytocompound through the intermolecular features like hydrogen bonds, electrostatic interactions, and other covalent and non-covalent bonds. The binding profiles are helpful to understand the novel active sites and its topology. Likewise, the identification of allosteric binding sites denotes the targeted or specific binding capacity of the ligand. Also, it is useful to study the key intermolecular interactions and perturbations that are induced by the novel compound during complex formation (Arcon et al. 2019).

By overlapping the phytocompounds with a known similar chemical entity can identify the unique structural features. Also, it allows us to understand how selectively phytocompounds bind on the receptor. SBDD is an iterative process, where the ligands are screened in series to select the best candidate out of the library. The other important point to emphasize is that the receptor flexibility greatly influences the result of novel ligand discovery. This is due to the fact that phytocompounds with heavy molecular weight and diverse structures are incompatible to enter the cavities of certain macromolecules. Upon docking analysis, molecular dynamics evaluation shows the deep network of interactions like hydrogen bonds, Van der Waals interactions, and electrostatics. Structural changes made by the natural compound can be assessed by the backbone root mean square deviation (RMSD). Also, it can find the perturbation of individual amino acid using root mean square fluctuation (RMSF) (Kumar et al. 2019).

25.3.3 Molecular Docking

Molecular docking is one of the most predominant methods in the drug discovery process, which is helpful to predict the accurate binding sites of a target for a given ligand (Meng et al. 2011). Both structure-based and ligand-based drug discovery methods depend on docking for evaluating the targeted receptors and identified and/or improved ligands. These techniques have gained more popularity due to several reasons, including low cost and efficient screening in less time. Docking algorithms virtually evaluate the receptor-ligand complex through the intermolecular interactions, and can also rank the ligand confirmations based on binding modes and

Table 25.2 Molecular docking software and the relevant algorithms used by the tool

Tool name	Algorithm/scoring function	License
GOLD	Genetic algorithm	Commercial
Glide	Exhaustive search	Commercial
AutoDock and AutoDock Vina	Lamarckian genetic algorithm, empirical scoring	Open
DOCK	Geometric matching	Free for academic
FlexX	Incremental build	Commercial
eHits	Exhaustive search	Commercial
Ligand fit	CHARMM force field-based docking	Commercial
ICM	Pseudo-Brownian sampling and local minimization	Commercial
HADDOCK	CPORT	Open

corresponding energies; various docking tools use pertinent algorithm to rank the complexes; a few are indexed in Table 25.2 (Ballante 2018).

Molecular docking process is constituted of two main steps: (1) firstly, the preparation of molecules, including prediction of the binding site or active site, protonation for the receptor and rotamers, and aromaticity setting for a ligand, and (2) secondly, a very crucial step of post-docking analysis, which includes predicting the accurate protein-ligand complex and its conformational energy. Docking is an iterative method in which the scoring algorithms evaluate the finest conformation at the smallest energy level by altering the structure according to the active site coordinates.

25.3.3.1 Conformational Analysis

Systemic search techniques encourage slight differences in the structural parameters and gradually alter the ligand's conformation (Zsoldos et al. 2007). The algorithm tests the conformation space's energy landscape and converges to the minimum energy solution, corresponding to the most probable binding mode after countless search and assessment cycles (Zsoldos et al. 2007). Though this conformational analysis is effective in discovering the conformational space, it is expected to get stuck at a local minimum and dissatisfy to identify the global minimum (Ferreira et al. 2015; Zsoldos et al. 2007). This shortcoming can be overcome by conducting simultaneous searches from different energy landscape points, i.e., separate conformations. Stochastic methods perform the conformational search by altering the ligand's structural parameters randomly. The algorithm creates a set of molecular conformations for this purpose and populates a broad variety of the energy landscape. Since the algorithm encourages a wide coverage of the energy landscape, the computational cost associated with this operation is relatively very less (Gorelik and Goldblum 2008). Systematic and stochastic methods are included in widespread molecular docking programs with particular solutions to their corresponding issues (Gorelik and Goldblum 2008). Systematic search techniques, for example, investigate all structural parameter combinations. As the degree of liberty associated with the ligand rises, the amount of possible combinations increases exponentially,

leading to a phenomenon known as a combinatorial explosion (Gorelik and Goldblum 2008). Docking programs like FRED, Surflex, and DOCK fix this issue by implementing an incremental building algorithm that gradually builds the ligand on the binding site (Jain 2003; McGann 2012). In this approach, the chemical structure is segregated into several distinct functional domains, and then desired components docked into the binding site complementary region. The algorithm carries out the conformational search only for the fragments added, reducing the degrees of freedom to be explored and thus avoiding combinatorial explosion (Ewing et al. 2001; Morris et al. 1996).

By implementing ideas of evolutionary theory and natural selection, the genetic algorithm (GA) addresses the elevated computational costs associated with stochastic methods, successfully implemented with protein-ligand docking (Guan et al. 2017). As a first step, the algorithm encodes in a chromosome, which is represented by a vector with all the structural parameters of the initial structure (Krovat et al. 2005). Starting from this chromosome, the random search algorithm generates an original population of chromosomes covering a wide region of the energy landscape (Krovat et al. 2005). This generated population is assessed, and the most adjusted chromosomes are chosen as templates for generating the next population (i.e., those with the smallest energy values) (Krovat et al. 2005). By transferring favorable structural features from one population to another, this method reduces the average energy of the chromosome ensemble, thus decreasing the conformation room to be investigated (Krovat et al. 2005).

25.3.3.2 Evolution of Binding Energetics

To assess the binding energy of the expected ligand-receptor complexes, molecular docking programs use scoring features (Foloppe and Hubbard 2006). The energy variation is caused by the binding constant (Kd) and the Gibbs free energy (GGL) owing to the creation of the ligand-receptor structure (Foloppe and Hubbard 2006). Binding energy prediction is carried out by assessing the most significant physical-chemical phenomena engaged in the binding of ligand receptors, including intermolecular interactions, desolvation, and entropic impacts (Huang et al. 2010). The higher the amount of physical and chemical parameters assessed, the more accurate the scoring function is (Huang et al. 2010). However, the computational cost increases in proportion to the number of factors included in the feature, a deficiency which decreases the docking algorithm's productivity (Kitchen et al. 2004). Ideally, effective scoring functions should provide a balance between precision and velocity, which is critical when working with big ligand sets. Scoring functions are classified in the following three groups: features based on force field, empirical, and knowledge-based (Murray et al. 1998). Force field scoring functions estimate the binding energy by summing in a particular master function the contributions of bonded (bond stretching, angle bending, and dihedral variation) and unbonded conditions (Murray et al. 1998). This type of scoring function uses an ab initio method to calculate the energy associated with each function term using the classical mechanic's equations. Their inaccuracy in estimating entropic contributions is a significant restriction of force field-based techniques (Murray et al. 1998). This

absence of a sensible physical model to describe this phenomenon is due to this shortcoming (Eldridge et al. 1997). In addition, the solvent is not explicitly regarded, which impedes the assessment of desolvation energies. Another sort of assessment technique is empirical scoring functions. Each function word defines one sort of physical case engaged in ligand-receptor complicated formation (Eldridge et al. 1997). These include relationships between hydrogen, ionic and apolar, and desolvation and entropic impacts. The weight constants generated by the statistical model are then used to adjust the equation circumstances as coefficients (Jain 2003). Their reliance on the precision of the information used to create the model is a drawback of empirical scoring functions (Jain 2003). However, empirical features are quicker than force field-based techniques due to the simplicity of the terms used for energy (Terp et al. 2001). Surflex and FlexX are broadly used molecular docking programs using empirical scoring functions (Terp et al. 2001).

The knowledge-based scoring functions are a third method used to assess ligand-receptor binding energy (Teramoto and Fukunishi 2007). The technique utilizes a pair of energy potentials obtained from known complexes of ligand and receptors to achieve a general function (Teramoto and Fukunishi 2007). These potentials are built by taking into consideration the frequency with which the structural data set contains two distinct atoms within a specified range (Mysinger and Shoichet 2010). Various kinds of interactions found in these individual interactions are provided in the final score. Since knowledge-based features do not depend on reproducing binding affinities (empirical methods) or ab initio calculations (force field methods), they provide an appropriate equilibrium between precision and velocity (Mysinger and Shoichet 2010).

25.3.3.3 Covalent Bonds in Molecular Docking

In several therapeutic fields such as cancer, diabetes, and infectious, cardiovascular, gastrointestinal, and neurological diseases, covalent medications have proven to be appropriate options (Kumalo et al. 2015). Recent studies have suggested that about one-third of the enzyme modulators presently on the market are covalent inhibitors (Kumalo et al. 2015). Covalent ligands act by inactivating their objectives irreversibly; therefore, restoring the inhibited biological function includes resynthesizing the targeted protein (Singh et al. 2011). Usually, covalent inhibitors bind with elevated affinity to their molecular objectives, resulting in a long-lasting pharmacological reaction and therefore requiring less frequent administration (Singh et al. 2011). However, well-known covalent drug disadvantages such as toxicity, absence of specificity, and elevated reactivity have resulted in most research and development programs avoiding such compounds (Singh et al. 2011).

This concept has been reconsidered, and there has lately been an enhanced interest in covalent inhibitors. As a consequence, various strategies for approaching the binding of covalent small molecule inhibitors have been created (Singh et al. 2011). Covalent docking algorithms are designed to investigate the ligand's energy landscape when it is covalently linked to the receptor and to assess the binding energy of the interaction (Ouyang et al. 2013). Despite the latest revival of covalent drugs, molecular modeling techniques designed to tackle the covalent

docking issue are not as advanced as non-covalent docking techniques (Ouyang et al. 2013). Covalent drug binding has some distinctions from non-covalent molecular interaction, particularly with regard to thermodynamic binding (Xu and Lill 2013). With excellent precision, non-covalent binding events can be predicted by current molecular mechanics (MM) algorithms (Xu and Lill 2013). These techniques, however, do not satisfactorily address the development of covalent bonds. Quantum mechanical (QM) techniques can properly handle the problem of covalent bond formation, which can investigate the entire mechanism of response. Molecular docking programs such as DOCK (Ewing et al. 2001), AutoDock (Morris et al. 1996), and GOLD (Jones et al. 1997) have targeted the issue of modeling covalent bonds in molecular docking (Verdonk et al. 2003). For covalent docking leadership, each of these programs utilizes a particular approach. For example, GOLD attempts to imitate the formation of covalent bonds by defining an atom as "link atoms" in both the ligand and the receptor (Verdonk et al. 2003). The ligand connection atom is then overlaid on the protein connection atom, and the covalent bond geometry is assessed by a particular scoring function. Another program, DOCKovalent, is a DOCK3.6 adaptation directed at large-scale, covalent virtual screening (Verdonk et al. 2003). The algorithm strategy describes a covalent attachment point and investigates the ligand conformational space around the covalent bond model (Verdonk et al. 2003). The default scoring feature introduced in DOCK3.6 ranks each conformation. Another strategy is the latest adaptation of AutoDock4, which proposes the technique of covalent docking called a two-point attractor (Verdonk et al. 2003). The default AutoDock routine comprises calculating an energy interaction map, built using multiple probe atoms, and a subsequent conformational search using these maps as reference tables for evaluating binding energy. The two-point attractor approach works as follows: first, the residue's two covalently bound terminal atoms are removed from the ligand. Next, this fragment is connected to the ligand's right atom and marked with two particular kinds of atoms (A and B) (Ferreira et al. 2015). A Gaussian function is then used to produce altered interaction maps for these atoms, focused in the residue of covalently bound amino acids on their initial place. These energy interaction maps penalize ligand conformations where A or B is not correctly positioned in their initial positions (Ferreira et al. 2015).

25.3.4 Molecular Dynamics

A key but often ignored element to be regarded in molecular docking is the flexibility of the target binding site (Lin 2011). During the molecular recognition process, enzymes and receptors can undergo conformational modifications (Salsbury 2010). These structural rearrangements are tiny in some cases, and the ligand fits into a binding site with low mobility (Salsbury 2010). Otherwise, some proteins conduct important conformation modifications that may involve secondary and tertiary components. The ligand usually stabilizes a subset of several feasible receptor conformations, shifting the balance to the minimum energy structures (Salsbury 2010). The ligand

usually stabilizes a subset of several feasible receptor conformations, shifting the balance to the minimum energy structures (Salsbury 2010). In such instances, MD simulations may generate alternative states of conformity corresponding to those structures induced by ligand (Durrant and McCammon 2011). Furthermore, if no appropriate crystallographic structures are accessible for a specific molecular target (i.e., structures with inaccessible or poorly defined binding locations), MD can be used to produce a number of useful docking structures. Potential conformational states are therefore sampled using MD simulations based on available crystallographic information, and affordable conformations (i.e., those with affordable and well-defined binding cavities) can be chosen for molecular docking (Durrant and McCammon 2011). In addition, MD can be used to assess the stability of a suggested molecular docking ligand-receptor complex (Durrant and McCammon 2011). If an MD-generated ligand conformation deviates from the respective docking solution by more than a specified RMSD value, the anticipated ligand-receptor complex may be regarded unstable (Harvey and De Fabritiis 2012). Newton's motion equations, as described in classical mechanics, are used in molecular dynamics to specify the position and speed of each atom in the system being studied (Nichols et al. 2011). As a consequence, it is possible to examine the trajectory and temporal evolution of a complex of ligand receptors (Chen et al. 2015). Initially, the atoms are assigned a particular setup with the purpose of reproducing the actual system's temperature and pressure (Nichols et al. 2011). The position and velocity of each of these atoms can be determined at a later time from the calculation of the forces acting on each particle. These calculations are conducted continuously until a specified moment interval integrates the molecular trajectories. The forces acting on the structure are determined by the potential for molecular interaction, which is generally parameterized by the calculation of quantum chemical or experimental information. This set of parameters (force field) determines the contribution to the overall function of each sort of interaction. Among the diverse available force fields, AMBER, CHARMM, and GROMOS (Christen et al. 2005) can be emphasized as commonly used in simulations of molecular dynamics. Notwithstanding its utility, MD has its constraints. Among them, we can stress the elevated computational cost of simulating big systems, which generally consists of thousands of atoms when complexes of ligand-receptors are being studied. Some of the conformational modifications made by receptors during molecular recognition happen on timescales that exceed the computational ability available. MD is able to make significant contributions to SBDD despite its constraints, particularly when coupled with other molecular modeling techniques, such as molecular docking (Borhani and Shaw 2012).

25.4 Conclusions

The use of therapeutic compounds of medicinal plants can be a potential treatment. Identification of novel bioactive compounds helps in developing new and potential drugs. Chemo- and bioinformatics are the reliable and cost-efficient approaches for understanding ligand-receptor interactions in this regard. Numerous software tools

are available to build, assess, and optimize the efficacy of the ligands as well as target molecules. On the other hand, along with numerous advantages, few shortfalls are also there in computational drug discovery. Since the biological systems are complex, biological activity and molecular functions indulge multiple macromolecule cascades, but computational drug discovery techniques cannot cover all the molecules at once. Taking together, future research should focus more on using computational biology to design new, effective, and more potent drugs for therapy with a better understanding of drug and receptor interaction.

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In Silico Molecular Docking of Glycyrrhizin and Breast Cancer Cell Line Proteins

26

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Contents

Abstract

Globally, breast cancer is one of the major cancers in females. The incident rates are increasing in recent years, particularly in developed countries. However, the lowest survival rates are found in less developed countries, because of the lack of

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specific symptoms at an early stage, inadequate diagnostic equipment, and lesser treatment facilities. Therefore, the development of a new cancer therapeutic approach remains the most challenging area in the medical field. Naturally derived products play a significant role in the discovery of novel drugs. It can be a potential treatment option for cancers. In this study, 3D (three-dimensional) structures of breast tumor cell proteins like p53 (a cellular cancer antigen), NF-kB (nuclear factor kappa B)-p105 subunits, and addressin, also known as MAdCAM-1 (mucosal addressin cell adhesion molecule 1) were generated, and their binding affinity with glycyrrhizin was determined through local docking. The proteins were constructed by SWISS-MODEL, and their physiochemical characters were assessed by ExPASy's ProtParam Proteomics server. After that, they were validated by PROCHECK, ERRAT, and Verify 3D programs. Lastly, the protein structures were docked successfully with glycyrrhizin using BSP-SLIM server. The binding energy between glycyrrhizin and p53, NF-kB-p105 subunits, and MAdCAM-1 were − 4.040, −5.127, and − 5.251 kcal/mol, respectively. The MAdCAM-1 had the strongest bond with glycyrrhizin, due to its lowest binding energy. Glycyrrhizin can be a potential drug candidate for cancer treatment. Thus, this protein model can further be validated in laboratory experiments to study its mechanisms of action.

Keywords
Modeling · p53 · Nuclear factor · Mucosal addressin · Active site · Ligand · Docking

26.1 Introduction

Worldwide, breast cancer is one of the leading diseases in females. It is escalating in recent years, particularly in the developed countries as compared to less developed countries. Breast cancer in many cases is detected in the late phases. In 2011, it was estimated that over 508,000 women in the world died, because of breast cancer based on the estimation of global health in 2013 (WHO 2013). Estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) positive, and triple negative or triple positive are the three main types of breast cancers. In about 20–25% of breast tumors, the cells make a high quantity of HER2 (Chiang and Butte 2009; Roy et al. 2016). In addition, 10–15% of females under the treatment of early breast cancers agonize a confined re-emergence, i.e., local recurrent breast cancers within 10 years. During this phase, it causes a substantial physical and mental illnesses, and most of the persons receiving cancer treatments die within 5 years of relapse (Roy et al. 2016). This is due to the poor prognosis, such as failure to observe definite symptoms in the initial phase of disease, causing postponements in diagnosis. Also, breast cancer has a destructive nature as proven by the extraordinary rate of metastasis or local spreading at the time of medical diagnosis. Moreover, the diagnostic practices are not specific and lack sensitivity and hence do not

support cancer screening effectively. However, the study on breast cancer has been limited in scope. Furthermore, studies have not addressed the issues of specificity and mechanisms of action. Currently, the available treatments for cancers, such as surgery, the use of chemotherapeutic drugs, and radiation therapy are not completely effective against breast cancer, thus having high incidences or little survival rate. Furthermore, these treatments cause negative side effects, such as liver failure, cardiomyopathy, and an increased risk of developing other types of cancer (Ashwini et al. 2017). Thus, the development of novel tonic approaches to breast cancer still remains as a thought-provoking field in cancer research.

Naturally derived bioactive compounds play a significant role in discovering novel lead molecules and support the drug industries. Over the past few decades, approximately 50% of the currently available medicines presented to the marketplace are mainly obtained from natural resources (Newman and Cragg 2012; Swamy et al. 2017). Cancer can be portrayed by the arrest of cell death, the restraint of apoptosis, and the quickened multiplication of cloned cells. The understanding of the systems of cell death execution and the part that they play in various infections opens new remedial procedures (Green and Kroemer 2004). As a part of cancer treatments, many natural products have been tried against numerous types of cancer cells (Capua et al. 2010; Santosh et al. 2018; Ravichandra et al. 2018). Many of the natural compounds have shown to be very effective against different types of cancers. Hence, there is an enormous interest among researchers in the area of natural products to identify unique medicinal plants, their bioactives, or bioactive compounds derived from natural sources for the development of cancer drugs (George et al. 2010; Jayaprakash et al. 2018; Akhtar and Swamy 2018). Glycyrrhizin is one of the bioactive components of *Glycyrrhiza glabra* (licorice) root. It has been widely utilized in various folk medicines for treating numerous diseases, such as bronchitis, gastritis, and jaundice. Previous studies described that glycyrrhizin has pharmacological effects as antiulcer, anti-inflammatory, antiviral, anticariogenic, and antispasmodic properties (Ramos-Tavor and Muriel 2019).

Computational biology tool is growing rapidly, because it is less expensive and time-consuming than experimental approaches. It is the best way to analyze the biological data. Molecular docking has been a standout among the most frequently utilized techniques in structure-based drug design (SBDD), due to its capability to anticipate with a substantial level of accuracy. Also, it offers the adaptation of small molecule ligands within a proper target site (Meng et al. 2011; Roy et al. 2016; Jayaprakash et al. 2018; Elengoe and Hamdan 2018). For example, a molecular docking analysis was carried out by taking 22 isolated compounds of *Vitex altissima* L. on the basis of their anticancer potential as reported from the comprehensive literature survey. The molecular docking study was performed with Argus Lab 4.0.1 to study on their binding interaction between 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and B-cell lymphoma 2 (BCL2) cells. The docking analysis proved that the active plant compound can be used as a candidate drug for treating cancer without any adverse side effects (Ali et al. 2017). Based on Roy et al. (2016) study, 12 compounds (apigenin, allicin, phloroglucinol, kaempferol, isobutyl isothiocyanate, quercetin, taurine, ferulic acid (FA), S-allyl cysteine (SAC), SACS, p CA, γGSAC, and S-allyl mercapto

cysteine) from garlic showed good interaction with the breast cancer targets. The binding energies of the compounds from garlic with the breast cancer targets were found to be ranging from −66.84 to −168.57 kcal/mol. According to De and De (2019), curcumin, capsaicin, rosmarinic acid, and 6-shogaol had good interaction with breast cancer targets. They had the highest C docker interaction energies.

The objectives of this study is to create 3D (three-dimensional) structures of breast cancer cell proteins, such as p53 (a cellular cancer antigen), NF-kB (nuclear factor kappa B)-p105 subunits, and addressin, also known as MAdCAM-1 (mucosal addressin cell adhesion molecule 1), and also to examine their binding affinities with glycyrrhizin using the local docking approach.

26.2 Methodology

26.2.1 Target Sequence

The complete amino acid sequences of cellular tumor antigen p53 (GI: 2491729), NF-kB-p105 subunits (GI: 109633022), and MAdCAM-1 (GI: 109633022) were obtained from the National Center of Biotechnology Information (NCBI) databank.

26.2.2 Homology Modeling

The 3D models of p53, NF-kB-p105 subunits, and MAdCAM-1 are unavailable or rarely reported. Therefore, the models were generated using SWISS-MODEL (Biasini et al. 2014) and visualized by the PyMOL software (Delano 2001).

26.2.3 Physiochemical Characterization of the Protein Models

The structural analysis of proteins was performed by the ExPASy's ProtParam Proteomics server as described by Gasteiger et al. (2005). The Color Protein Sequence (Colorseq) study as previously followed by Prabi (1998) was utilized to detect the number of polar and nonpolar residues. The software ESBRI was carried out to identify the salt bridges in the protein models (Costantini et al. 2008), while the number of disulfide bonds was calculated by making use of the Cys_Rec program (Roy et al. 2011).

26.2.4 Secondary Structure Prediction Analysis

The secondary structural characteristics of p53, NF-kB-p105 subunits, and MAdCAM-1 were predicted from SOPMA (Self-Optimized Prediction Method from Alignment) (Geourjon and Deleage 1995).

26.2.5 Validation of Protein Models

The structures of proteins were analyzed with PROCHECK by Ramachandran plot investigation (Laskowski et al. 1993). Further, these models were analyzed by making use of the Verify 3D (Eisenberg et al. 1997), ERRAT (Colovos and Yeates 1993), ProQ (Wallner and Elofsson 2003), and ProSA programs (Wiederstein and Sippl 2007).

26.2.6 Identification of Active Sites

To identify the binding sites of the cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1, they were submitted into active site prediction server—SCFBio (Jayaram 2014).

26.2.7 Preparation of Ligand

The tertiary structure of the glycyrrhizin was not publicly available. The complete sequence of glycyrrhizin was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and the structure was made available in sdf format. Later, it was converted to pdb format by using molecule file format conversion (via ChemAxon JChem).

26.2.8 Protein-Ligand Docking

The three-dimensional models of cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 were docked with glycyrrhizin using the BSP-SLIM server (Hui and Yang 2012).

26.3 Results and Discussion

26.3.1 Physiochemical Characterization

The complete amino acid sequence of cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 was found to be 393, 968, and 382, respectively. The mucosal addressin cell adhesion molecule 1 had the lowest molecular weight of 43653.18 Da, while NF-kB-p105 subunits had the highest molecular weight of 105356.00. The computed isoelectric point (pI) value for all three protein models was indicated as basic, because the value was found to be less than 7 ($pI < 7$). The factors, such as the molecular weight and isoelectric point, were used to setup the simulation box. These factors will affect the stability of protein models. A total of 50 negatively charged residues (Arg + Lys) and 46 positively charged residues (Arg + Lys) for cellular tumor antigen p53 were observed. The NF-kB-p105 subunits had a total of 133 negatively charged residues and 93 positively charged residues. Likewise, about 40 negatively charged residues and 23

positively charged residues for mucosal addressin cell adhesion molecule 1 were recorded. The extinction coefficient was calculated using the extent of light being absorbed by a protein at a particular wavelength based on tyrosine, tryptophan, and cysteine residues. These residues play a valuable role by cross-linking proteins, which increases the rigidity of proteins and also functions to confer proteolytic resistance (Gasteiger et al. 2005). The extinction coefficient for cellular tumor antigen p53 and mucosal addressin cell adhesion molecule 1 was 31,970 M/cm; however for NF-kB-p105 subunits, it was found to be 60,740 M/cm. ExPASy's ProtParam instability index for cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 was found to be 73.59, 38.15, and 63.75, respectively. Based on the ExPASy's ProtParam instability index, NF-kB-p105 subunit protein was classified as stable, as the uncertainty index rate was observed to be lesser than 40. The other two protein models were considered to be unstable. These proteins require more steps to converge the protein during the energy minimization period (Roy et al. 2016; Jayaprakash et al. 2018). Further, the aliphatic index for cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 was 59.08, 84.74, and 84.27, respectively. Lastly, an average of hydropathicity (GRAVY) value for cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 was −0.756, −0.339, and − 0.250, respectively. The summary of the result is stated in Table 26.1.

Furthermore, cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 had more polar residues (47.33%, 43.70%, and 41.36%) than nonpolar residues (24.94%, 33.42%, 31.61%, and 31.94%). This was determined using Color Protein Sequence analysis. In this study, the ESBRI was used to identify salt bridges, which were formed by arginine residues. Cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 had 18, 11, and 20 salt bridges, respectively. Arginine is effective in overpowering the aggregation of proteins and could be useful during the purification process. This shows that the MAdCAM-1 was the most stable protein among all the models. The results of Cys_Rec are shown in Table 26.2. Cys_Rec analysis is used to determine the number of disulfide bonds in the protein models.

Table 26.1 Physiochemical character of cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 as predicted by ExPASy's ProtParam program

Protein	Length	Molecular weight	pI	−R	+R	Extraction coefficient	Instability Index	Aliphatic index	GRAVY
Cellular tumor antigen p53	393	43653.18	6.33	50	46	31,970	73.59	59.08	−0.756
NF-kB-p105 subunits	968	105356.00	5.20	133	93	60,740	38.15	84.74	−0.339
Mucosal addressin cell adhesion molecule 1	382	40155.28	5.00	40	26	31,970	63.75	84.27	−0.250

26.3.2 Secondary Structure Prediction of Protein Models

Results of SOPMA (view) analysis revealed that alpha helix dominated among secondary structure elements followed by random coils, extended strand, and beta turns for all the breast cancer cell line proteins (Table 26.3). Helix structures enhance the stability of the protein models. Furthermore, cellular tumor antigen

Table 26.2 The presence of disulfide (ss) bond predicted by Cys_Rec server

Protein	Cys_Rec	Score
Cellular tumor antigen p53	Cys_47	−67.8
	Cys_116	−60.0
	Cys_148	−66.7
	Cys_163	−68.7
	Cys_170	−44.6
	Cys_184	−57.9
	Cys_220	−58.3
	Cys_264	−59.4
NF-kB-p105 subunits	Cys_61	−44.9
	Cys_87	−60.8
	Cys_118	34.5
	Cys_123	48.4
	Cys_161	−60.1
	Cys_261	−32.9
	Cys_272	−35.6
	Cys_446	−0.3
	Cys_666	−38.2
	Cys_703	−37.8
	Cys_925	−24.1
Mucosal addressin cell adhesion molecule 1	Cys_47	56.0
	Cys_51	61.7
	Cys_94	50.9
	Cys_98	46.7
	Cys_134	24.7
	Cys_204	42.1
	Cys_345	−21.6

Table 26.3 Secondary structure of the cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1

Secondary structure	α Helix	Extended strand	Beta turn	Random coil
Cellular tumor antigen p53	18.58	18.58	8.91	54.96
NF-kB-p105 subunits	32.54	18.39	9.75	37.60
Mucosal addressin cell adhesion molecule 1	28.27	14.40	6.28	51.05

p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 consist of 4, 32, and 15 helices, respectively. The longest and shortest helixes of proteins are described in Table 26.4.

26.3.3 Validation of Protein Models

Cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 protein models were validated through Ramachandran plot calculations using PROCHECK program for stereo chemical quality and geometry of protein (Fig. 26.1). The Ramachandran plot for the cellular tumor antigen p53 protein demonstrated that 82.5% were situated in the most favored area, 15.7% in the additionally allowed area, 1.3% in the generously allowed area, and 0.4% in the disallowed area. The stability of the cellular tumor antigen p53 protein was assessed to be very good among other protein models. The PROCHECK analysis shows that cellular tumor antigen p53 and mucosal addressin cell adhesion molecule 1 in most allowed area were more than 80% but NF-kB-p105 subunits scored marginally lower than 80% which was 78.6 for the most favored area (Table 26.5). PROCHECK analysis revealed that several residues such as ALA 119 and ASN 311 were situated out of the energetically favored regions of Ramachandran plot for cellular tumor antigen p53 while ALA 176, SER 73, GLN 52, ARG 191, and SER 223 residues were located at the disallowed region for NF-kB-p105 subunits. However, there were no residues found at the disallowed region for mucosal addressin cell adhesion molecule 1. Levitt-Gerstein (LG) and MaxSub scores were determined using ProQ. The scores for cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin were 2.843, 5.977, and 4.212, respectively. MaxSub scores were 0.304 (p53), 0.404 (kappa), and 0.348 (mucosal) (Table 26.5). All protein structures were in the acceptable range to create a good model.

ERRAT value depends on the statistics of nonbonded atomic interactions in the three-dimensional protein structure. The normally accepted range is more than 50% for a high-quality protein model. In this study, the ERRAT score for mucosal addressin cell adhesion molecule 1 which is 86.928 was the highest (Fig. 26.2). This analysis confirmed that mucosal addressin cell adhesion molecule 1 structure had a very high-quality model compared with other protein models. The ERRAT scores

Table 26.4 Composition of α helix in cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1

Protein	Volume	Residues forming pocket		
Amino acid	Longest α helix	Residues	Shortest α helix	Residues
Cellular tumor antigen p53	α_8	16	α_5	2
NF-kB-p105 subunits	α_{20}	26	α_2, α_3, α_7	1
Mucosal addressin cell adhesion molecule 1	α_{13}	15	α_4, α_5, α_{10}	1

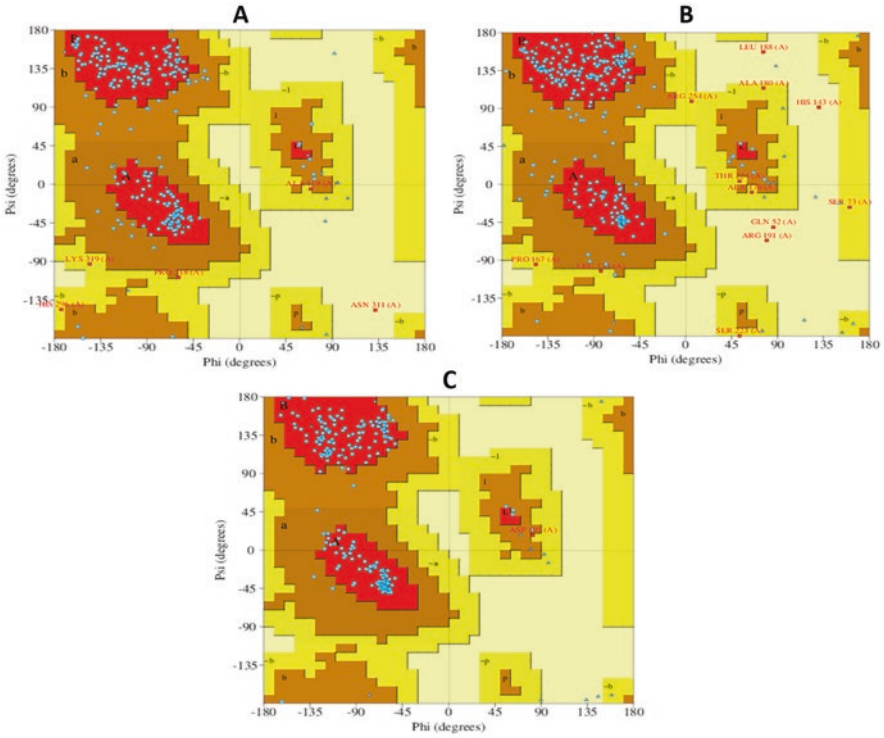

Fig. 26.1 Ramachandran plots for (**a**) cellular tumor antigen p53, (**b**) NF-kB-p105 subunits, and (**c**) mucosal addressin cell adhesion molecule 1 were generated through PROCHECK. PROCHECK shows the residues in most favored (red), additionally allowed (yellow), generously allowed (pale yellow), and disallowed regions (white color)

Table 26.5 Validation of the cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 using PROCHECK program

	Ramachandran plot statistics (%)				Goodness factor			ProQ	
Structure	Most favored	Additionally allowed	Generously allowed	Disallowed	Dihedral angles	Overall forces	Overall average	LG score	Max Sub
p53	82.5	15.7	1.3	0.4	−0.23	−0.18	−0.19	2.843	0.304
NF-kB-p105 subunits	78.6	17.3	2.6	1.5	−0.46	−0.09	−0.30	5.977	0.404
Mucosal addressin cell adhesion molecule 1	82.2	10.7	5.3	1.8	−0.68	−0.39	−0.53	4.212	0.348

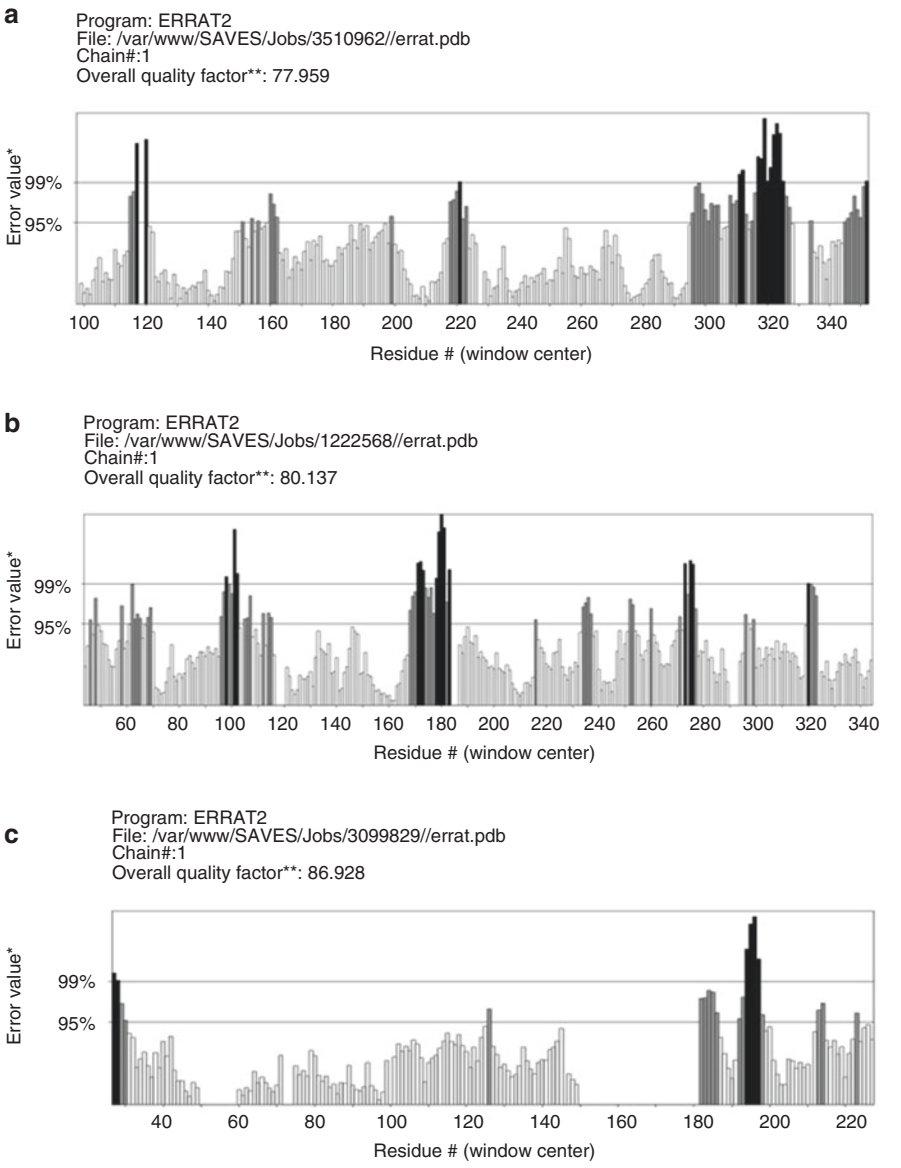

Fig. 26.2 ERRAT plot for (**a**) cellular tumor antigen p53, (**b**) NF-kB-p105 subunits, and (**c**) mucosal addressin cell adhesion molecule 1

for cellular tumor antigen p53 and NF-kB-p105 subunits were 77.959 and 86.928, respectively.

Verify 3D server verified that cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell 1 had 71.49%, 96.81%, and 95.69% of the residues. All the proteins had an average 3D-1D score of more than 0.2, which shows that they

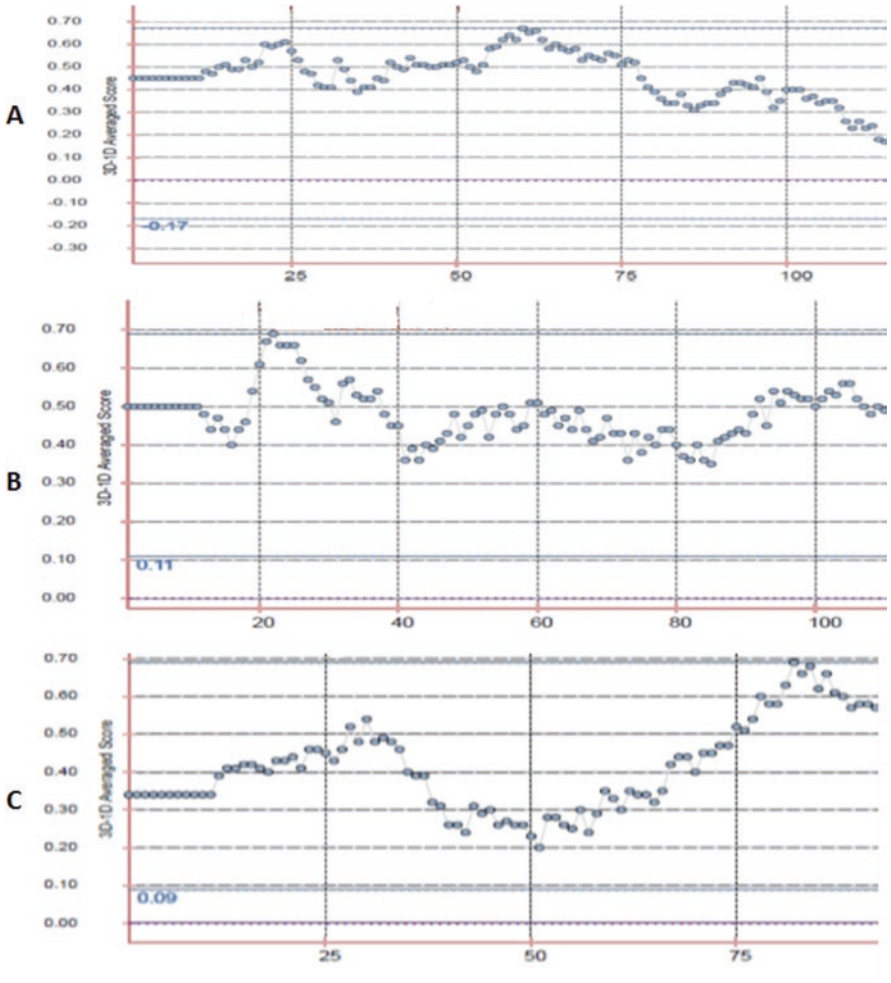

Fig. 26.3 Verify 3D plot of (**a**) cellular tumor antigen p53, (**b**) NF-kB-p105 subunits, and (**c**) mucosal addressin cell adhesion molecule 1

were good protein models (Fig. 26.3). The cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell attachment particle 1 protein models satisfied the criteria for the protein stability. Therefore, all the proteins were further analyzed for docking approach.

26.3.4 Identification of Active Sites

The size, protein volume of active site, and residues shaping a pocket of active site for cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell

Table 26.6 Predicted active sites of the cellular tumor antigen p53, NF- B-p105 subunits, and mucosal addressin cell adhesion molecule 1

Protein	Volume	Residues that forming pocket
Cellular tumor antigen p53	1534	TYR10, GLN11, ASP135, CYS136, GLY15, PRO157, LEU157, PHE16, ILE162, ARG17, ARG174, ASN175, SER176, PHE177, GLU178, LEU18, RG189, LY19, RG190, LU192, LU193, EU196, RG197, YS199, LY200, LU201, RO202, IS203, HIS204, LU205, LEU206, RO 207, RO208, LEU21, HIS22, LEU255, YS258, GLN261, ER28, YS31, YR33, RO35, ALA36, LEU37, SN38, YS39, MET40, PHE41, YS42, YS48, RO49, LN51, RP53, SP55, LN7, YS71, YS8, THR9
Nuclear factor NF-kappa-B-p105 subunits	1311	ALA116, RG117, HR119, LU120, LA121, YS122, LE123, RG124, LY125, TYR126, SN127, RO128, LY129, EU130, AL132, LA137, YR138, EU139, LN140, LA141, LU142, LY143, LY144, LY145, SP146, RG147, ET176, HR178, HE180, EU181, RO182, SP183, ER184, HR185, LY186, ER187, HE188, HR189, RG190, RG191, EU192, LU193, RO194, AL195, RO53, ALA54, LYS55, ILE57, LN59, LEU60, VAL61, LEU69, HIS70, HIS78, GLU80 ASP81, LE83, YS84, HR85, HR87
Mucosal addressin cell adhesion molecule 1	938	LYS116, HR118, RO119, AL12, AL120, SP121, RO122, SN123, LA124, EU125, HE127, RO144, LU145, AL146, LN147, LU148, LU149, LU150, LU157, SP158, AL159, LA16, EU160, HE161, AL163, EU17, LY18, RG187, EU188, RO189, EU41, HR43, EU45, ER63, EU64, ER65, LA66, LA67, LY68, HR69, RG70, LN86, EU87, EU88, AL89, YR90, HE92, PRO93, SP94

molecule 1 were determined using active site prediction server—SCFBio (Table 26.6). The protein volume of cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 volume is 1534, 1311, and 938 A^3, respectively.

26.3.5 Protein-Ligand Docking

According to Pusphalatha et al. study (2017), negative values of glide score that ranges between −8.05 and − 12.1 and glide energy between −43.21 and − 56.46 for curcumin showed a stronger hydrogen bonding with higher lipophilicity. This indicated that a superior contact was established at the active sites of cancer proteins (HER2, human estrogen receptor, epidermal growth factor tyrosine protein kinase C-SRC, ERBB2, and HSP90 proteins), leading to the inactivation. As reported by Ashwini et al. (2017), five plant compounds, such as coumarin, camptothecin, epigallocatechin, quercetin, and gallic acid, were shown to have a good interaction with caspase 3-HeLa protein. The highest binding energy and negative value (−378.3 KJ/mol) were obtained for coumarin-caspase 3 protein interaction. Gallic acid with caspase 3 had the lowest negative value (−181.3 KJ/mol). The docking score was determined through Hex 8.0.0 docking software. Khan et al. study (2013) describes that NF-κB precursor protein p105 had good interaction with quercetin and 1-caffeoylquinic acid. 1-Caffeoylquinic acid and quercetin function as

Table 26.7 Docking result of the cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1

Protein	Binding energy (kcal/mol)
Cellular tumor antigen p53	−4.040
NF-kB-p105 subunits	−5.127
Mucosal addressin cell adhesion molecule 1	−5.251

inhibitors due to binding energy of −11.50 and − 12.11 kcal/mol, respectively. These plant compounds may be potential drugs for cancer treatment. According to Chen et al. (2012), myricetin docked with PI3-K, MEK1, and Raf proteins successfully. They had binding scores of −9.40, −8.86, and − 7.73 kcal/mol, respectively. This plant compound inhibits the activity of kinase in cancer pathway. Based on Jayaprakash et al. (2018), the highest docking scores were obtained when alginic acid was docked against β-actin giving a docking score of −8.9081 kcal/mol followed by caspase 3 (−8.5634 kcal/mol) and caspase 9 (−8.2513 kcal/mol). Moreover, fucoidan was docked with caspase 9; it showed the highest docking score of −8.5091 kcal/mol, followed by caspase 3 (−7.6683 kcal/mol) and β-actin (−7.6636 kcal/mol). In this study, cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 were effectively docked with glycyrrhizin using the BSP-SLIM server. The best docking orientation was chosen based on the negative and lowest energy value (DG_{bind}). Docking analysis demonstrated that there was a strong bond between mucosal addressin cell adhesion molecule 1 and glycyrrhizin which was −5.251 kcal/mol (Table 26.7). The binding energy was calculated as the sum of the intermolecular energy and torsion energy. Thus, the results clearly indicate that glycyrrhizin can be an effective agent in the treatment of cancer.

26.4 Conclusion

In conclusion, cellular tumor antigen p53, NF-kB-p105 subunits, and mucosal addressin cell adhesion molecule 1 were successfully docked with glycyrrhizin. The three protein models had strong interaction between glycyrrhizin, due to their negative binding energy. Therefore, in vitro and in vivo investigations of in silico interpretations of this protein-ligand model will help in designing a more potent structure-based drug. In the future, more research activities are required to validate glycyrrhizin as a potential drug candidate for cancer treatment. In addition, this protein model can further be validated in laboratory experiments to study its mechanisms of action.

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